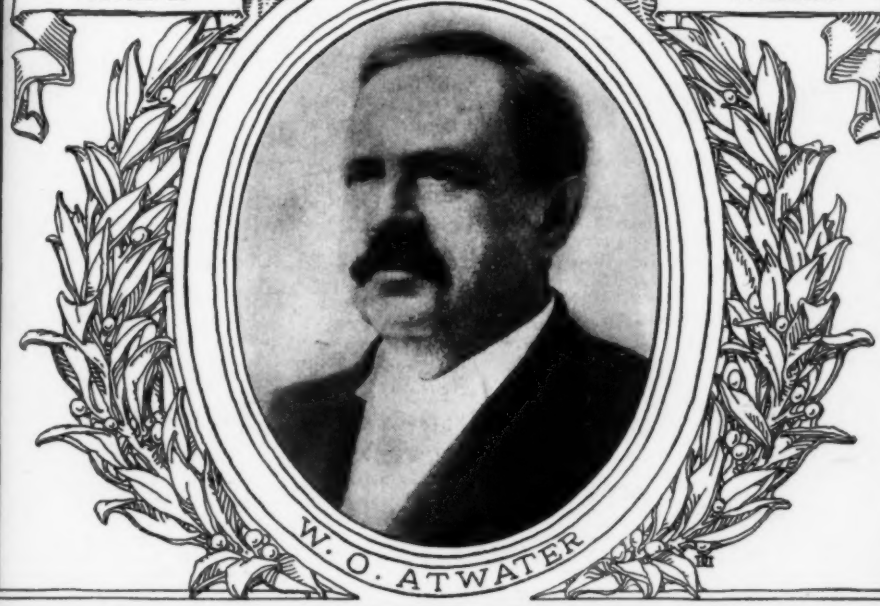
SEPTEMBER 10, 1943

THE JOURNAL OF NUTRITION

VOLUME 26 NUMBER 3



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PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA 4, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

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DIGESTIBILITY OF CERTAIN HIGHER SATURATED FATTY ACIDS AND TRIGLYCERIDES

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(Received for publication February 25, 1943)

INTRODUCTION

In view of the rather wide differences which the writers ('40) have found between the digestive coefficients of certain fats, it has seemed desirable to determine the digestibility of several pure saturated fatty acids and the corresponding triglycerides. Digestion tests have been conducted with mature male rats with diets containing different percentages of pure stearic, palmitic, myristic, and lauric acids and with pure tristearin, triplamitin, trimyristin, and trilaurin.

PREVIOUS INVESTIGATIONS

In experiments with dogs, Arnschink (1890) found tristearin to be 9–14% digestible. Nearly all of the undigested stearin was present in the feces as the triglyceride. Lyman ('17) obtained digestive coefficients of approximately 95% for glyceryl palmitate and 82% for palmitic acid. He states that the melting point of an ester is not the only, and probably not the chief, factor determining the rate of hydrolysis and absorption.

MATERIAL AND METHODS

Stearic, palmitic, myristic, and lauric acids and the corresponding triglycerides, each of the highest purity obtainable, were purchased from a firm specializing in pure organic chemicals. Pure virgin olive oil was also purchased. The oil was stored at about 4°C.

THE JOURNAL OF NUTRITION, VOL. 26, NO. 3
SEPTEMBER, 1943
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The percentage composition of the diets which were fed to the rats was as follows: Diet 1 — casein 18.6; salts 4.0; dextrose 75.4; yeast vitamins 2.0; and no fat. Diet 2 — casein 20.0; salts 4.3; dextrose 68.7; yeast vitamins 2.0; and fat 5.0. To 1 kilo of each diet was added 100 ml. of cod liver oil concentrate.[1]

EXPERIMENTAL PROCEDURE

Each diet containing 5% of a mixture of olive oil and a definite proportion of a fatty acid or triglyceride was fed to eight mature male rats for 10 days. After a preliminary period of 3 days, the feces were collected for 7 days from each rat and the quantity of feed consumed by each was determined. Each rat was then changed to a fat-free diet and, after a preliminary period of 3 days, the feces were collected for 7 days. The feces were dried to constant weight at 100°C. and the fat content was determined as previously described by the writers ('42). When the diet contained fatty acids, the digestive coefficient was calculated as the percentage of the total fatty acids. The digestive coefficient was calculated for each of the eight rats on each diet, but only the average value is reported.

EXPERIMENTAL RESULTS

The results of the digestion tests with two lots of olive oil and with mixtures of olive oil and different proportions of stearic, myristic, and lauric acids are shown in table 1. The melting points of the pure fatty acids and of the mixtures are shown also. Two lots of olive oil were used, as the first was insufficient.

The results of the tests with the mixtures of olive oil and saturated fatty acids show that stearic acid depressed the digestibility of the mixture only slightly less than the proportion of stearic acid present. The digestive coefficients for stearic acid alone have been calculated on the assumption that the reduction in digestibility was due entirely to unabsorbed stearic

[1] 100 ml. of an ethereal extract from saponified cod liver oil was added to 1 kg. of the diet and the mixture was agitated until the ether had evaporated.

TABLE 1

Digestibility by male rats of mixtures of certain saturated fatty acids and olive oil when the diets contained 5% of fat.

DESCRIPTION OF FAT MIXTURE	MELTING POINT OF PURE FATTY ACIDS	MELTING POINT OF MIXTURE	DIGESTIVE COEFFICIENT OF MIXTURE		CHANGE IN DIGESTIBILITY COMPARED WITH OLIVE OIL	CALCULATED DIGESTIBILITY OF PURE FATTY ACIDS
			Average [1]	Range		
	°C.	°C.	%	%	%	%
Olive oil no. 4085 [2]			97.4	94.1– 99.3		
Olive oil no. 4095			99.5	99.1–100.0		
Stearic acid 5%, olive oil 95%	69	43	92.6	90.5– 95.5	– 4.8	9.4
Palmitic acid 5%, olive oil 95%	63	37	94.2	91.9– 96.6	– 3.2	39.6
Myristic acid 5%, olive oil 95%	53		99.1	97.6–100.0	+ 1.7	
Lauric acid 5%, olive oil 95%	44		98.0	97.3– 99.0	+ 0.6	
Stearic acid 10%, olive oil 90%		51	88.3	85.0– 91.2	– 9.1	13.3
Palmitic acid 10%, olive oil 90%		44	90.8	87.6– 94.2	– 6.6	37.1
Myristic acid 10%, olive oil 90%		32	97.6	95.0– 99.9	+ 0.2	
Lauric acid 10%, olive oil 90%			98.3	97.1– 99.7	+ 0.9	
Stearic acid 15%, olive oil 85%		55	85.0	79.4– 89.5	– 12.4	21.0
Palmitic acid 15%, olive oil 85%		48	86.6	82.4– 89.8	– 10.8	31.2
Myristic acid 15%, olive oil 85%		39	97.1	93.8–100.0	– 0.3	
Lauric acid 15%, olive oil 85%			97.2	96.6– 98.5	– 0.2	
Stearic acid 25%, olive oil 75%		59	76.5	71.1– 80.1	– 20.9	19.6
Palmitic acid 25%, olive oil 75%		53	77.6	75.0– 82.4	– 19.8	23.8
Myristic acid 25%, olive oil 75%		44	92.7	88.4– 97.0	– 4.7	81.9
Lauric acid 25%, olive oil 75%		32	92.6	82.5– 96.3	– 4.8	81.5
Stearic, palmitic, myristic, and lauric acids, 2.5% each; olive oil 90%			93.5	90.4– 97.0	– 6.0	

[1] Each digestive coefficient represents the average value obtained with eight rats.

[2] Olive oil no. 4085 was used in all mixtures except the last one.

acid. To obtain these figures, the percentage of fatty acid in each mixture was calculated as the percentage of the total fatty acids, since the digestive coefficients were calculated on that basis.

The results of the digestion tests with the mixtures of palmitic acid and olive oil show that each mixture had a somewhat higher digestive coefficient than a similar mixture of stearic acid and olive oil. The approximate digestive coefficients for palmitic acid alone were calculated in the same manner as for stearic acid.

Myristic and lauric acids were very thoroughly absorbed when the percentage of acid in the mixture did not exceed 15%; but when the proportion was increased to 25%, the digestibility was materially lower. The approximate digestive coefficients for myristic and lauric acids alone are shown only for the highest level of intake.

The digestibility of the mixture of 2.5% each of stearic, palmitic, myristic, and lauric acids and olive oil no. 4095 was lower than might have been expected from the results previously reported in table 1. This mixture had a digestive coefficient six points lower than the olive oil used although the mixture contained only 2.5% each of stearic and palmitic acids.

The results of the digestion tests with the mixtures of triglycerides and olive oil are shown in table 2. The results with tristearin show that this triglyceride, whether present singly or together with other triglycerides in a mixture with olive oil, depressed the digestibility of the fat nearly as much as the percentage of tristearin present. When the mixtures contained 5 and 10% of tristearin, the approximate digestive coefficients of the tristearin alone were calculated on the assumption that the reduction in digestibility was due entirely to unabsorbed tristearin.

The results of the tests with the mixtures containing tripalmitin indicate that this triglyceride was much more digestible than tristearin. The approximate digestive coefficients for tripalmitin alone, when the mixtures contained 5 and 10% of this triglyceride, are shown.

TABLE 2

Digestibility by male rats of mixtures of certain saturated triglycerides and olive oil when the diets contained 5% of fat.

DESCRIPTION OF FAT MIXTURE	MELTING POINT OF TRIGLYCERIDE	MELTING POINT OF MIXTURE	DIGESTIVE COEFFICIENT OF MIXTURE		CHANGE IN DIGESTIBILITY COMPARED WITH OLIVE OIL	CALCULATED DIGESTIBILITY OF TRIGLYCERIDE
			Average [1]	Range		
	°C.	°C.	%	%	%	%
Olive oil no. 4085 [2]			97.4	94.1– 99.3		
Olive oil no. 4095			99.5	99.1–100.0		
Tristearin 5%, olive oil 95%	70	55	92.7	91.3– 94.4	– 4.7	6.0
Tripalmitin 5%, olive oil 95%	65	45	95.8	91.2– 99.3	– 1.6	84.0
Trimyristin 5%, olive oil 95%	56		96.4	93.5– 99.8	– 1.0	
Trilaurin 5%, olive oil 95%	48		98.2	94.8– 99.9	+ 0.8	
Tristearin 10%, olive oil 90%		59	89.2	88.0– 91.3	– 8.2	8.0
Tripalmitin 10%, olive oil 90%		51	95.6	92.9– 97.2	– 1.8	82.0
Trimyristin 10%, olive oil 90%		42	99.0	97.8–100.0	+ 1.6	
Trilaurin 10%, olive oil 90%			99.9	99.5–100.0	+ 2.5	
Tristearin 5%, trimyristin 5%, olive oil 90%		54	95.3	93.9– 96.8	– 4.2	
Tristearin 5%, trilaurin 5%, olive oil 90%		53	95.2	91.1– 96.6	– 4.3	
Tripalmitin 5%, trimyristin 5%, olive oil 90%		42	96.3	93.7– 98.4	– 3.2	
Tripalmitin 5%, trilaurin 5%, olive oil 90%		40	97.7	96.6– 98.5	– 1.8	
Tristearin, tripalmitin, trimyristin, and trilaurin 2.5% each; olive oil 90%			96.9	94.1– 98.6	– 2.6	

[1] Each digestive coefficient represents the average value obtained with eight rats.

[2] Olive oil no. 4085 was used in the first two series of digestion tests and sample no. 4095 was used in the third series.

The mixtures containing 5 or 10% each of trimyristin or trilaurin were as digestible as the olive oil alone. Under these conditions each triglyceride was practically 100% digestible.

A comparison between the results obtained with the fatty acids and those with the corresponding triglycerides indicates that both tristearin and stearic acid were very poorly absorbed, whereas tripalmitin appeared to have been much better absorbed than palmitic acid. Myristic and lauric acids and the corresponding triglycerides were utilized equally well.

SUMMARY

Experiments were conducted with mature male rats to determine the digestibility of pure stearic, palmitic, myristic and lauric acids and of the corresponding triglycerides. Each fatty acid was mixed in the proportions of 5, 10, 15, and 25% with pure olive oil, and each triglyceride was mixed in the proportions of 5 and 10%. The fat mixture constituted 5% of the diet. The following results were obtained:

Stearic acid was very poorly absorbed at each level of intake, the approximate digestive coefficients ranging from 9.4 to 21%.

Palmitic acid was utilized somewhat more efficiently than stearic acid, the approximate digestive coefficients ranging from 23.8 to 39.6%.

Myristic and lauric acids were practically 100% digestible when the fat mixtures contained 5, 10, or 15% of either acid, but the digestibility was somewhat lower when the mixture contained 25% of acid.

Tristearin was very poorly utilized, the approximate digestive coefficients being 6 and 8% when the fat mixtures contained 5 and 10% of the triglyceride.

Tripalmitin was much more digestible than tristearin, the approximate digestive coefficients being 84 and 82% when the fat mixtures contained 5 and 10% of the triglyceride.

Trimyristin and trilaurin were very thoroughly absorbed.

LITERATURE CITED

ARNSCHINK, L. 1890 Versuche über die Resorption verschiedener Fette aus dem Darmkanale. Z. Biol., vol. 26, pp. 434–451.

HOAGLAND, RALPH, AND GEORGE G. SNIDER 1940 Nutritive properties of certain animal and vegetable fats. Tech. Bull. 725, 12 pp., U. S. Department of Agriculture.

———— 1942 Nutritive properties of lard and other shortenings. Tech. Bull. 821, 12 pp., U. S. Department of Agriculture.

LYMAN, J. F. 1917 Metabolism of fats. I Utilization of palmitic acid, glyceryl palmitate, and ethyl palmitate by the dog. J. Biol. Chem., vol. 32, pp. 7–11.

THE EFFECT OF COOKING WITH AND WITHOUT SODIUM BICARBONATE ON THE THIAMINE, RIBOFLAVIN, AND ASCORBIC ACID CONTENT OF PEAS[1]

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(Received for publication February 20, 1943)

Sodium bicarbonate has been employed in the cooking of vegetables, largely because of the decreased cooking time required and because its use is attended by retention of the normal color of green vegetables. On the other hand, the idea has become fairly general among nutritionists that the addition of sodium bicarbonate will result in a large destruction of certain members of the B complex (chiefly thiamine and riboflavin) as well as of vitamin C. In fact, the current publicity campaign for better nutrition has stressed that the use of soda should be avoided in cooking vegetables.

Halliday ('32a) found that the thiamine in protein-free milk was 70 to 80% destroyed by cooking for 1 hour at a pH of 10, and completely destroyed by heating for 4 hours at this pH. The rate of destruction was much less at a pH of 4.3 or 7.0 where the maximum loss was 40%. Lantz ('38) found as much as 50% loss in the thiamine of Pinto beans soaked 16 hours in distilled water following by cooking 2¾ hours. Somewhat larger losses occurred when a 0.5% solution of sodium bicar-

[1] Some of these data have been reported at a meeting of the Society of Experimental Biology and Medicine at California Institute of Technology on January 21, 1943. The thiamine data have been summarized in Science, vol. 97, p. 50 (1943). The work was made possible through a grant from Church and Dwight Company, Inc.

bonate was used for the preliminary 16-hour soaking period although the results on the bioassay group receiving 0.2 gm. of dried peas actually were considerably better with the soda group. It is also uncertain how much loss of thiamine can be traced to the procedure used in drying the beans, as Mickelsen, Waisman and Elvehjem ('39) have found that an appreciable destruction of thiamine occurs in drying even when a vacuum is employed if the temperature exceeds 70° C. Aughey and Daniel ('40) also have found a slightly greater destruction of thiamine in peas cooked in water alone and in water containing sodium bicarbonate, 11% of the original thiamine being in the water in both cases while 80% and 67%, respectively, were retained in the peas. An identical time of cooking (12 minutes) was used in both instances.

Although riboflavin is extremely thermostable, it is especially sensitive to light. Conner and Straub ('41) and Williams and Chedelin ('42) have demonstrated the rapid destruction of this vitamin by sunlight. Halliday ('32b), in experiments carried out with the thiamine tests by an identical plan described earlier, also showed that the destruction of riboflavin was somewhat greater when heated at a pH of 10 than obtains at a pH of 4.3 or even at one of 7.0. Lantz ('39) found that the availability of riboflavin in Pinto beans is influenced somewhat by the method of cooking which included the use of sodium bicarbonate, but the differences were not great.

While it has been recognized for a long time that vitamin C is somewhat thermolabile, it is now evident that the most rapid destruction occurs when oxidation is brought about in the presence of copper or silver salts. However, Sherman ('41) in discussing the effect of soda in cooking, states the following about the extent of destruction:

> "Not necessarily all of it; but always more of the vitamin C is destroyed when soda is added than when it is not. The more soda added the more vitamin destroyed; this is true whether or not the change in pH caused is such as to shift it over the neutral point of pH = 7".

The present tests were designed to get additional data on the relative destruction of thiamine, riboflavin and vitamin C of peas when cooking was carried out with water alone or with water to which sodium bicarbonate was added. The earlier data are not entirely applicable to the cooking of fresh vegetables as pH values as high as 10 will not occur when the quantities of sodium bicarbonate used in cooking are employed; moreover, most of the earlier investigators have made comparisons of the effect of cooking with and without soda on the basis of identical times of cooking rather than on the periods required to complete the cooking under the two procedures.

METHODS OF ANALYSIS

Thiamine was determined by a modification of the yeast fermentation method of Schultz, Aiken, and Frey ('42). This procedure was changed by weighing the water forced over from the gasometers into bottles during the course of the fermentation rather than by reading off the volumes. Also as containers for the reaction mixtures, 250 ml. centrifuge tubes connected with the tubes to the gasometers by spherical joints were found more convenient and to obviate leakage. The reliability of the above method of analysis was confirmed by comparing the thiamine contents of peas dried at 60°C. in a vacuum oven after cooking with water alone or water containing sodium bicarbonate as determined by the yeast fermentation method and the bioassay procedure of Kline, Hall and Morgan ('41).

Riboflavin was estimated by the microbiological method of Snell and Strong ('39). In order to obtain the full response, digestion with takadiastase apparently is unnecessary, as identical results were obtained with and without the preliminary treatment. That our results represented riboflavin is evident from the fact that activity of the pea suspension was completely destroyed by photolysis.

The technic of Loeffler and Ponting ('42) was used for the determination of vitamin C. Not only were uniform results

obtained when vitamin C was determined promptly after preparation of pea suspensions, but also it was demonstrated that ascorbic acid added to such suspensions could be quantitatively accounted for.

Peas were cooked in a covered aluminum saucepan or in a similar pyrex dish with a cover. In these experiments, 85 gm. of peas were cooked with 180 ml. of water. When sodium bicarbonate was used, 0.22 gm. was added. In preliminary tests the periods of time required for peas to become tender, when cooked in water alone or water plus sodium bicarbonate, were determined. The periods required for fresh peas were 17 and 8 minutes, respectively, while with frozen peas, the values were 6 and 4 minutes. In all cases the peas were added to the water after it had been brought to a boil.

After cooking, the peas were filtered from the cooking water, transferred to a Waring blender, fresh water added and the peas macerated to a fine susupension. Analyses were made both on the cooking water and on the suspension of the peas. The extent of retention of the green color in the peas was established by comparing the color of the mashed pulp with standard color plates (Maerz and Paul, '30). The pH was determined by the use of the Beckman pH meter.

The peas were purchased in various local markets. The frozen peas consisted of one variety where plate freezing was employed and several varieties where tunnel freezing was used.

RESULTS

A summary of the results on the thiamine content of peas as influenced by the method of cooking is given in table 1.

The thiamine content of the raw fresh peas averaged 333 μg. per 100 gm. while that of the two types of frozen peas gave mean values of 408 and 351 μg. These fall within the values listed by Sherman ('41). The addition of sodium bicarbonate increased the pH of the water somewhat over 1 pH unit, the values found after cooking being between 8.70 and 8.84. In the frozen peas prepared by tunnel freezing and in the fresh peas, identical quantities (about 80%) of the original thiamine

were retained after cooking the peas either with or without sodium bicarbonate. However, slightly greater losses were incurred in the thiamine which had been leached into the cooking water when soda was employed, but the destruction only amounted to 8 and 4% of the total amount present in the frozen and fresh peas, respectively.

TABLE 1

The thiamine contents of frozen and fresh peas before and after cooking in water alone or water containing sodium bicarbonate.

TYPE OF PEAS AND METHOD OF COOKING	NO. OF TESTS	PH OF WATER AFTER COOKING	THIAMINE IN MICROGRAMS PER 100 GM.			PER CENT RETAINED IN PEAS	PER CENT LOST
			In peas	In cooking water	Total		
Frozen type I [1]							
Uncooked	3				408		
Water-cooked	3	7.66	326	90	416	79.8	0
$NaHCO_3$-cooked	3	8.77	330	44	374	80.8	8.3
Frozen type II [1]							
Uncooked	1				351		
Water-cooked	1		238	102	340	67.8	3.1
$NaHCO_3$-cooked	1	8.70	193	25	218	55.0	37.9
Fresh							
Uncooked	4				333		
Water-cooked	4	7.29	257	78	336	77.2	0
$NaHCO_3$-cooked	4	8.84	258	63	321	77.4	3.6
Fresh (overcooked)							
Uncooked	1				185		
Water-cooked	1	7.50	67	34	101	36.3	45.4
$NaHCO_3$-cooked	1	9.38	59	19	78	31.9	59.6

[1] Type I represents a brand of peas prepared by tunnel freezing; and type II one prepared by plate freezing.

In peas frozen by the plate process where many of the cell membranes were ruptured, the loss was greater in the peas cooked with sodium bicarbonate. The thiamine retained in the peas cooked in water was 68% while that remaining in those cooked with sodium bicarbonate was only 55%. Approximately 38% of thiamine was lost in the soda-cooked peas and this was largely due to the greater destruction of that leached into the

water. In another series of tests when fresh peas were overcooked and many of the cell membranes ruptured, the loss of thiamine also was greater than in the earlier tests. Only somewhat over 30% of the original thiamine was retained in the peas and the loss amounted to 45 and 60% in the water- and $NaHCO_3$-cooked samples, respectively.

Identical results were found for the thiamine content of dried peas as determined by the fermentometer method and by bioassay. Thus, the average thiamine content for the dried peas which had been cooked in water was 1050 and 1070 μg. per 100 gm., respectively, when determined by the fermentometer method and by bioassay. The results on the peas which had been cooked in sodium bicarbonate were 1345 and 1330 μg., respectively. This would indicate that no biologically inactive products which stimulate yeast growth and are not destroyed by sodium sulfite are formed in peas during cooking with water alone or with water and bicarbonate.

The results of cooking on the riboflavin content of fresh and frozen peas are given in table 2.

The riboflavin content of the various samples of frozen peas varied from 171 to 200 μg. per 100 gm., while the limits of values for fresh peas were practically identical (168 to 203). These values are slightly lower than the figures reported by Sherman ('41) where the limits are given as 200 to 250 μg.

In all cases 64 to 70% of the riboflavin remained in the peas after cooking, and the figures are identical regardless of method of cooking. Practically no destruction of vitamin B_2 occurred with the fresh peas and only a slight decrease in the frozen peas regardless of the procedure used in cooking. No differences in the riboflavin content of peas cooked in the dark (aluminum pan) and in the light (pyrex dish) were noted.

The effects of cooking on the ascorbic acid content of fresh peas and three types of frozen peas are reported in table 3.

The ascorbic acid content of the fresh peas averaged 23.7 mg. per 100 gm., which is in the upper range given by Sherman ('41). The values on frozen peas are consistently lower. It is possible that this may be due to the lower content of vitamin

TABLE 2

The riboflavin content of fresh, frozen, and dried cooked peas as affected by cooking with water alone and with added sodium bicarbonate.

TYPE OF PEAS AND METHOD OF TREATMENT	NO. OF TESTS	TYPE OF CONTAINER IN COOKING	RIBOFLAVIN IN MICROGRAMS PER 100 GM.			PER CENT RETAINED IN PEAS	PER CENT LOST
			In peas	In cooking water	Total		
Frozen type I [1]							
Uncooked	1				171		
Photolyzed [2]	1				0		100
Uncooked	1				189		
Water-cooked	1	Pyrex	122	42	164	64.8	13.1
$NaHCO_3$-cooked	1	Pyrex	127	46	163	67.0	13.8
Uncooked	1				200		
Water-cooked	1	Aluminum	141	46	187	70.5	6.5
$NaHCO_3$-cooked	1	Aluminum	141	45	186	70.5	7.5
Fresh peas							
Uncooked	1				168		
Takadiastase [3]	1				170		
Uncooked	2				203		
Water-cooked	2	Pyrex	132	73	205	65.0	0.0
$NaHCO_3$-cooked	2	Pyrex	131	73	204	64.6	0.0
Water-cooked	2	Aluminum	130	68	198	64.1	2.5
$NaHCO_3$-cooked	2	Aluminum	135	65	200	66.6	1.5
Dried peas [4]							
Water-cooked	1	Pyrex			468		
$NaHCO_3$-cooked	1	Pyrex			492		

[1] Type I represents a brand of peas prepared by tunnel freezing, and type II one prepared by plate freezing.

[2] An alkaline solution was subjected for 8 hours to the light from a 75-watt lamp at 30 cm.

[3] 0.1 gram of takadiastase (Merck) was added to pea suspension; they were incubated for 3 hours, autoclaved, diluted and added to tubes for assay.

[4] Dried peas used in bioassay test on thiamine. Before drying, these had been cooked with water or water containing $NaHCO_3$ as in other tests.

TABLE 3

The ascorbic acid content of fresh and frozen peas as affected by cooking with water alone and with added sodium bicarbonate.

TYPE OF PEAS AND METHOD OF TREATMENT	NO. OF TESTS	ASCORBIC ACID IN MG. PER 100 GM.			PER CENT RETAINED IN PEAS	PER CENT LOST
		In peas	In cooking water	Total		
Frozen peas, type I [1]						
Uncooked (brand 1)	1			20.6		
Uncooked (brand 2)	1			19.8		
Uncooked (brand 1)	1			18.1		
Water-cooked	1	11.0	4.3	15.3	60.7	15.5
$NaHCO_3$-cooked	1	11.0	3.7	14.7	60.7	18.8
Uncooked (brand 2)	1			18.0		
Water-cooked	1	10.1	4.8	14.9	56.2	17.2
$NaHCO_3$-cooked	1	10.3	4.0	14.3	57.2	20.5
Frozen peas, type II [2]						
Uncooked [3]	3			12.2		
Uncooked [4]	1			18.2		
Water-cooked	1	8.2	7.3	15.5	45.1	14.8
$NaHCO_3$-cooked	1	8.1	7.4	15.5	45.0	14.8
Fresh peas						
Water-cooked [5]	1	12.9	13.7	26.6		
$NaHCO_3$-cooked	1	15.0	11.2	26.2		
Uncooked	3			23.7		
Water-cooked	3	11.6	10.3	21.9	48.9	7.6
$NaHCO_3$-cooked	3	12.9	10.5	23.4	54.4	1.3

[1] Frozen by tunnel process.

[2] Frozen by plate process.

[3] Frozen peas were allowed to thaw before being weighed and only peas with intact skins were used.

[4] Frozen peas weighed without thawing.

[5] No value on uncooked peas.

C in the fresh peas from which the frozen peas were prepared; on the other hand, it may be that some vitamin C is destroyed in the preparation for freezing or in the storage. It is well-known that peas prior to freezing are treated with boiling water for short periods after mechanical shucking to remove undesirable residues. Because of the readier solubility of vitamin C than of thiamine or riboflavin, it is possible that some is leached out by this treatment. The proportion of ascorbic acid remaining in the pea after cooking varies from 45 to 60%. No greater leaching out or destruction of the vitamin C obtains in the peas cooked in water containing bicarbonate of soda than in those cooked in water alone.

The greater retention of the green color in peas cooked with sodium bicarbonate was indicated from the deeper green color of the pulp. After cooking in water the color compared with plate 20, no. 1 or 3, L, while after the use of sodium bicarbonate the pulp matched plate 19, 5 or 6, L, which is a greener shade, but contains less gray (Maerz and Paul, '30).

DISCUSSION

The addition of sodium bicarbonate in the proportion of 0.22 gm. (1/16 teaspoonful) to 180 ml. of water (1 cup) resulted in a decrease in the time required for cooking fresh peas (from 17 to 8 minutes) and frozen peas (from 6 to 4 minutes). There was only slightly greater destruction of thiamine under these conditions when sodium bicarbonate was added and this was due to the greater loss in that leached out of the peas; however, the amount still remaining in the peas at the conclusion was the same by both methods of cooking. On the other hand, the loss was greater when fresh peas were overcooked to the extent that they started to fall apart and in one sample of frozen peas in which the peas had been partially crushed in the processing. Both with water alone and with water containing sodium bicarbonate, much smaller quantities of thiamine remained in the peas.

The losses of riboflavin were under 15% in all tests and in four of the six series they were practically zero. There was no

greater destruction in the soda-cooked series than in those cooked in water alone; moreover, the retention of riboflavin in the peas, which varied from 64 to 70%, was similar when both methods of cooking were employed.

Vitamin C in general rather than thiamine or riboflavin was found to be more readily soluble in the cooking water, the amounts retained in the peas varying from 61 to 45%. The total destruction of the ascorbic acid was usually under 20% and here again there was no difference in the two methods of cooking. The destruction was much less in the fresh peas than in the frozen samples. In the former case only 1% destruction resulted in the peas cooked in sodium bicarbonate, while a loss of 8% was found in those boiled in tap water.

One reason why these results do not show the destructive effect of sodium bicarbonate that is reported in some of the earlier studies, is that the alkalinity employed was less. Whereas Halliday used a pH of 10, the values obtained in our tests were usually under a pH of 9. Secondly, the time of cooking used by some investigators has been the same for the sodium bicarbonate tests and those where water alone was employed. One of the chief advantages in the use of soduim bicarbonate is the shorter cooking time that its addition makes possible. In these tests the time necessary for cooking the peas sufficiently tender to be appetizing has been employed. Although thiamine, riboflavin and ascorbic acid are undoubtedly more thermolabile at the higher pH values, this tendency is compensated by the shorter cooking time possible when sodium bicarbonate is added. Also if the same length of cooking time is used with and without sodium bicarbonate, there is likely to be a greater destruction in the latter case because the peas begin to disintegrate. Any time when the intact membrane of the pea becomes ruptured with or without sodium bicarbonate, a markedly increased destruction of these water-soluble vitamins results.

The loss by leaching into the cooking water is least with thiamine, somewhat more with riboflavin and greatest with ascorbic acid. This may be because the two B vitamins are

largely non-diffusible since they are combined with protein while ascorbic acid is almost entirely free. The ready leaching of ascorbic acid may possibly account for the lower value found in frozen, as compared with fresh, peas.

The destruction of the thiamine, riboflavin, and ascorbic acid is undoubtedly considerably retarded by the buffering effect of the pea proteins. Also the cell membranes probably prevent any appreciable diffusion of the sodium bicarbonate to the inside of the cell. The protective effect on the peas is shown by the far greater destruction which occurs when 12.5 mg. of ascorbic acid are cooked with 160 ml. of water at various pH levels; when cooked for 17 minutes, 46.8% was destroyed in tap water, 13.6% at a pH of 4.3, and 58.2% in sodium bicarbonate for 8 minutes.

Further experiments are necessary to establish how general may be the application of the results obtained herein. It would seem advisable to re-investigate the destructive effect of sodium bicarbonate in the cooking of a wide variety of vegetables, making use of the more accurate procedures now available for the determination of the vitamins.

SUMMARY

The effect of cooking fresh and frozen peas in water alone and in water containing sodium bicarbonate on the thiamine, riboflavin and vitamin C content has been studied.

It has been found that the addition of 0.22 gm. of $NaHCO_3$ to 180 ml. of water in the cooking of 85 gm. of fresh peas lowers the time necessary for cooking from 17 to 8 minutes; of frozen peas, the decrease is from 6 to 4 minutes.

The following average values were found per 100 gm. of fresh peas: thiamine, 333 μg.; riboflavin, 168 to 203 μg.; and vitamin C, 23.7 mg. In frozen peas the values were similar with thiamine (351 to 408 μg.) and riboflavin (171 to 200 μg.), but were somewhat lower with ascorbic acid (12.2 to 20.6 mg.).

Approximately 80% of the thiamine, 65% of riboflavin, and 45 to 60% of ascorbic acid were retained in the peas after

cooking. No differences resulted from the addition of sodium bicarbonate to the cooking water.

When the vitamin content remaining in the peas was added to that of the cooking water, the recovery of these three vitamins was found to be practically complete. Similar results were obtained with respect to frozen peas, as far as thiamine and riboflavin are concerned. However, the destruction of vitamin C amounted to between 15 and 20% with the frozen peas, and this was not influenced by the method of cooking.

When fresh peas and one brand of frozen peas which were frozen by the plate process were overcooked to the extent that they started to disintegrate, the losses of thiamine from the peas were considerably greater and the recovery was much less. Under these conditions the destructive effect of sodium bicarbonate was found to be somewhat greater.

LITERATURE CITED

AUGHEY, E., AND E. P. DANIEL 1940 The effect of cooking on the thiamin content of foods. J. Nutrition, vol. 19, p. 285.

CONNER, R. T., AND G. J. STRAUB 1941 Combined determination of riboflavin and thiamin in food products. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 385.

HALLIDAY, N. 1932a Effect of heat at varying concentrations of hydrogen ion on vitamin B (B_1) in protein-free milk. J. Biol. Chem., vol. 98, p. 707.

———— 1932b Effect of heat at varying concentrations of hydrogen ion on vitamin G (B_2) in protein-free milk. J. Biol. Chem., vol. 95, p. 371.

KLINE, O. L., W. L. HALL AND J. F. MORGAN 1941 An improved rat growth method for the assay of vitamin B_1 including sulfite treatment of dietary consituents. J. Ass. Off. Agr. Chem., vol. 24, p. 147.

LANTZ, E. M. 1938 Effect of different methods of cooking on the vitamin B content of Pinto beans. New Mexico Agr. Exp. Sta. Bull, No. 254.

———— 1939 Effect of cooking on the riboflavin and vitamin B_1 content of Pinto beans. New Mexico Agr. Exp. Sta. Bull., No. 268.

LOEFFLER, H. J., AND J. D. PONTING 1942 Ascorbic Acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. Ind. and Eng. Chem., Anal. Ed., vol. 14, p. 846.

MAERZ, A., AND M. R. PAUL 1930 A Dictionary of Color, 2nd ed. McGraw-Hill Book Co., Inc., New York.

MICKELSEN, O., H. A. WAISMAN AND C. A. ELVEHJEM 1939 The distribution of vitamin B_1 (thiamin) in meat and meat products. J. Nutrition, vol. 17, p. 269.

SCHULTZ, A. S., L. AIKEN AND C. N. FREY 1942 Determination of vitamin B_1 by yeast fermentation method. Ind. and Eng. Chem., Anal. Ed., vol. 14, p. 35.

SHERMAN, H. C. 1941 Chemistry of Food and Nutrition. 6th ed. Macmillan Company, New York.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. and Eng. Chem., Anal. Ed., vol. 11, p. 346.

WILLIAMS, R. R., AND V. H. CHEDELIN 1942 Destruction of riboflavin by light. Science, vol. 96, p. 22.

THE B VITAMINS IN HONEY[1]

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(Received for publication March 25, 1943)

Honey is a widely used food — undoubtedly due to its sweetness, pleasant taste and aroma. Since honey is a natural substance and is food for the honeybee, there has been much speculation as to its value in the nutrition of man. Honey is prepared from the nectar of flowers by worker bees and stored in the honeycomb. Although honey is derived primarily from nectaries of flowers, we cannot overlook the part the honeybee plays in storing the nectar in the hive and in processing it to its final form. Thus honey should be considered the product of the bee processed from a plant product.

The analysis of honey has shown that it consists of approximately 75–80% sugar, mainly invert sugar, which is a conversion product of sucrose. Schuette et al. ('32, '37, '38, '39) have shown the presence of mineral elements in honey. Notable among these are iron, copper, sodium, potassium, manganese, calcium, magnesium, and phosphorus, all of which have been shown to be essential to good nutrition of animals. Since honey is basically derived from the flower and moreover is a food for the bee, much speculation has arisen as to its vitamin content, especially because great emphasis has been placed on the importance of vitamins in human nutrition. It seems that this problem has interested workers for two decades, many of whom have reported the absence of vitamins A, B, C, D, and E

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the American Honey Institute. The authors are indebted to Dr. Frank M. Strong for valuable suggestions regarding the microbiological assays.

in honey. These results may be interpreted in the light of the methods employed, which have been much improved in recent years.

Recently chemical determinations of vitamin C in honey have been made by Griebel ('38). He reported 160–280 mg. ascorbic acid per 100 gm. of mint honey and 7–22 mg. per 100 gm. of other honeys. Griebel and Hess ('39) identified the strong reducing substance in thyme and mint honeys as ascorbic acid. They reported 311.2 mg. and 102.6 mg. of this vitamin per 100 gm. of the respective honeys. Buckwheat honeys contained from 7.36–18.6 mg. ascorbic acid per 100 gm. They (Griebel and Hess, '40) determined also the ascorbic acid content of the nectar of the above plants. It was shown that there is a considerable loss of vitamin C when nectar is converted into honey. Schuette and Kaye ('42) investigated a series of honeys and found 0.55–20.9 mg. of vitamin C per 100 gm. The degree of destruction of vitamin C in honey was also studied. It was noted that there was a decrease in the total ascorbic acid content of honey after pure ascorbic acid was added. The loss was greatly increased in the honeys placed in diffused light.

Haydak and Palmer ('42 a) reported the vitamin content of honeys of varied nectar source and origin. They reported the following values per 100 gm. of honey: thiamine, 2.1–9.1 μg.; riboflavin, 35–145 μg.; pyridoxine, 210–480 μg.; pantothenic acid, 25–192 μg.; nicotinic acid, 4–94 mg.; ascorbic acid, 0.6–5.4 mg.

The authors of this paper will attempt to give a clear picture of the vitamins in honey obtained by the improved methods of vitamin assay. These include the more recent michrochemical and microbiological methods, some of which have been modified in the University of Wisconsin. Preliminary investigations did not reveal the presence of the fat-soluble vitamins; therefore only the contents of water-soluble vitamins were determined. Together with numerous samples of honey we have analyzed pollen and royal jelly, two natural substances also serving as food for bees.

EXPERIMENTAL

The honeys under investigation were obtained from the collection in the Laboratory of Foods and Sanitation of the Chemistry Department of the University of Wisconsin, from local grocery stores, and from beekeepers in various localities of the United States. Many of these honeys were received in the comb and extracted in the laboratory by straining through layers of cheesecloth. The wax was washed with distilled water and dried for further analysis.

The nicotinic acid assays were made by the microbiological method of Snell and Wright ('41). Samples were prepared as follows: 25 gm. of honey were diluted to 75 ml. with distilled water, autoclaved for 15 minutes at 15 pounds pressure, and then diluted to the necessary volume. It was found that aqueous extraction gave the same results as acid or alkali. The latter tended to produce excessive caramelization of the sugars in the honey. The effect of the large percentage of sugar on the assay method and also the effect of caramelization were investigated. The sugar that was being introduced by the sample of honey had no apparent effect on the assay, but the caramelization which resulted from acid or alkali treatment interfered very much with the determination of the end-point in the titration of the lactic acid produced. Recovery of added nicotinic acid ranged from 90% to 110%.

Pantothenic acid was determined by the microbiological method of Strong, Feeney and Earle ('41). Preparation of the sample was carried out as follows: 25 gm. of honey were diluted to 75 ml. and neutralized to pH 7.0 with NaOH. It was autoclaved for 15 minutes at 15 pounds pressure and diluted to the necessary volume. Enzyme digestion of the honey with clarase did not produce a significant change in the results. Ether extraction of the diluted honey did not remove any stimulatory or inhibitory substances. Recovery of added pantothenic acid ranged from 90% to 110%. Values are expressed in micrograms of calcium pantothenate per 100 gm.

Riboflavin was determined by the microbiological method of Snell and Strong ('39). Preparation of the sample was as follows: 25 gm. of honey were diluted to 75 ml. with distilled water and autoclaved for 15 minutes at 15 pounds pressure. The use of acid was avoided for the reasons explained for the nicotinic assay. Enzyme digestion with clarase did not alter the value significantly. It should be mentioned that the natural pH range of honey is 4–5; therefore the honey dilution was acid during the autoclaving, thereby reducing any possible destruction of the riboflavin. Ether extraction of the honey dilution did not remove any stimulatory or inhibitory substances.

Thiamine was determined by the Hennessy-Cerecedo thiochrome method as modified by the Research Corporation Committee on the Thiochrome Method (Hennessy, '42). A Coleman photofluorometer was used to measure the fluorescence of the thiochrome produced by the oxidation of the thiamine. This instrument can accurately determine 0.02 μg. of thiamine. Recovery of added thiamine ranged from 85% to 105%. Values are expressed in micrograms of thiamine-hydrochloride.

Pyridoxine was determined by the microbiological method of Atkin, Schultz, Williams, and Frey ('43). In this method the response of a strain of yeast to pyridoxine is measured by turbidity readings. The range of sensitivity of the assay is 5 to 40 mμg. Enzyme digestion was used to release any bound pyridoxine. Recovery of added pyridoxine ranged from 95% to 105%. Values are expressed in micrograms of pyridoxine-hydrochloride per 100 gm.

Biotin was determined by the microbiological method of Snell, Eakin, and Williams ('40), using yeast and measuring the growth of the organism by turbidity readings. The assays were conducted by Miss Josephine Gardner of the Biochemistry Department of the University of Wisconsin.

Folic acid was determined by the method of Mitchell and Snell ('41), using the streptococcus lactis organism. The assays were conducted by Mr. T. D. Luckey of the Biochemistry Department of the University of Wisconsin. The folic acid

values are expressed in this paper as micrograms of folic acid based on a solubilized liver standard with a potency of 40,000.

The samples of pollen which were analyzed were obtained from Professor Farrar of the Department of Economic Entomology of the University of Wisconsin. One sample of pollen was a mixture from an area around Madison, Wisconsin. The other sample was a mixture of pollen from Baton Rouge, Louisiana. The sample of royal jelly was obtained in a dry form from Dr. H. B. Parks of the Texas Agricultural Experiment Station.

RESULTS AND DISCUSSION

Table 1 gives the results of analyses of forty samples of honeys from different regions of the United States. Although the source of the honey is also listed, it is understood that honey is usually a mixture of nectars. The floral source listed, however, is the predominant one. An examination of the values obtained for the five members of the B-complex showed great variation among the samples. The greatest variation was in the nicotinic acid content. In one instance, a sample of honey contained nine times as much nicotinic acid as another. The least variation occurred in the thiamine and pyridoxine values, but even here the variation was as much as fivefold. An attempt was made to correlate the vitamin content with floral source, origin, and color-grade. This proved fruitless for we did not have a sufficiently large number of samples of honey of one locality to enable us to say that one set of data is typical of that source. The wide variation of the vitamin contents precluded any attempt to correlate the values with the color-grade of the honeys.

Table 2 presents a summary of the vitamin content of the honeys examined. It also shows the differences in the vitamin content of the old honeys as compared with the new samples. In the course of the investigation for pantothenic acid, it became apparent that the pantothenic acid values for a large number of honeys were lower than those of the rest. It was found that the group of honeys that had been obtained from

TABLE 1

Summary of vitamin assays of honey.

All values are in micrograms per 100 gm.

ORIGIN	FLORAL SOURCE	RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID	THIAMINE	PYRIDOXINE
Alabama	cotton	60	360	590	12.0	17.5
Arkansas	holly blossom	27	60	140	1.4	...
California	alfalfa	50	16	240	6.2	8.4
California	alfalfa	25	20	110	2.9	6.4
California	mesquite	14	24	93	3.5	...
California	orange blossom	9	9	96	2.5	6.3
California	sage	40	60	100	4.6	9.2
California	spikeweed	10	15	88	2.6	8.3
California	tamarisk	24	9	105	4.3	10.2
California	thistle	45	19	137	3.3	...
California	white sage	13	9	80	3.4	5.3
California	white sage	12	17	120	2.4	...
California	wild buckwheat	20	17	181	4.2	14.0
California	wild sage-buckwheat	21	17	135	4.2	10.2
Florida	orange blossom	30	60	95	3.8	7.0
Florida	tupelo	20	27	104	5.5	3.9
Florida	tupelo	15	13	100	3.0	8.9
Georgia	Mexican clover	15	13	75	2.2	7.8
Georgia	titi	18	20	63	2.9	4.4
Illinois	orange blossom	28	27	110	4.8	14.2
Illinois	sweet clover	20	46	80	3.4	5.5
Illinois	unknown	11	9	110	2.2	...
Iowa	sweet clover	22	40	110	4.2	9.6
Louisiana	white dutch clover	32	110	176	3.9	27.7
New York	buckwheat	24	63	123	4.2	12.6
Ohio	mixed flowers	35	116	110	5.5	11.7
Ohio	sweet clover	12	50	90	3.9	7.2
Texas	horse mint	64	12	600	4.3	...
Utah	sweet clover	20	32	72	2.8	4.6
West Virginia	sweet clover	19	32	150	4.2	8.2
Wisconsin	sweet clover	7	61	225	5.5	12.2
Wisconsin	sweet clover	20	65	98	2.8	5.2
Wisconsin	sweet clover	23	38	100	3.4	9.0
Wisconsin	sweet clover	19	52	89	2.5	5.7
Wisconsin	sweet clover	14	20	270	4.4	5.0
Wisconsin	white and sweet clover	15	35	85	5.6	10.6
Wisconsin	white and sweet clover	12	57	95	3.8	8.9
Wisconsin	white and sweet clover	33	48	200	4.2	7.5
Unknown	orange blossom	30	20	119	2.2	6.4
Unknown	sweet clover	30	44	75	3.7	4.6

the Chemistry Department contained 50% less pantothenic acid than those received from various beekeepers. The former were of the crops of 1935–1939 and had been stored in a dark closet at room temperature. The honeys obtained this year were of the crops of 1940–1942. Many of these samples were received in the comb; others had been extracted before shipment. The difference in the pantothenic acid content of these two groups may be explained by the fact that this vitamin is unstable in an acid solution. Honey is naturally acid, having a pH range of 4–5. This acidity is not very great, but any

TABLE 2

Comparison of aged and new honeys.

All values are in micrograms per 100 gm.

		RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID	THIAMINE	PYRIDOXINE
Honeys of years 1935–1939	Mean	21.8 ± 3.0	20.4 ± 2.4	124.4 ± 11.6	3.5 ± .25	7.6 ± .66
	Range	9 — 64	9 — 60	63 — 600	1.4 — 6.2	4 — 14
Honeys of years 1940–1942	Mean	26.3 ± 2.1	54.4 ± 3.6	108.5 ± 9.1	4.4 ± .50	10.0 ± 1.1
	Range	7 — 60	20 — 360	72 — 590	2.2 — 12	4 — 27

pantothenic acid that might be in the honey could be destroyed slowly over a period of years. This is of little significance to the beekeeper or the housewife who do not keep honey for long periods of time.

A few honey samples were analyzed for their biotin and folic acid content, two new members of the B-complex whose importance in nutrition is being investigated. Traces of biotin and folic acid were found, with the average values of 0.066 μg. and 3 μg. per 100 gm., respectively. Since the amounts found were very small as compared to other foods, we did not examine many honeys.

Table 3 presents a comparison of the vitamin content of the samples of honey of the years 1940–1942 with pollen and royal jelly. It may be noted that the royal jelly, a milky-white sub-

stance secreted by the worker bee is unusually high in pantothenic acid and biotin. This may be related to the rapid metabolism in the young bee larvae which depend on this substance for sustenance the first 3 days after emergence from the egg. Comparison of the vitamin content of pollen with that of honey revealed that in general pollen was a hundred times richer in the B vitamins than honey. This suggested that the vitamins in the honey may be due partially to the pollen present in each sample. All natural honeys contain a certain amount of pollen, which at times is very small in quantity. Haydak and Palmer ('42a) have found that a commercial process of clarifying

TABLE 3

Comparison of honey with pollen and royal jelly.

All values are in micrograms per gram.

	NO. OF SAMPLES	PANTOTHENIC ACID	RIBOFLAVIN	NICOTINIC ACID	THIAMINE	PYRIDOXINE	BIOTIN	FOLIC ACID
Honey	19	0.55	0.26	1.1	0.044	0.10	0.00066	0.03
Pollen	2	27.0	16.7	100.0	6.0	9.0	0.25	..
Royal jelly	1	320.0	28.0	111.0	18.0	10.2	4.1	0.5

honey reduces the vitamin contents 33–50% of the original values. When we filtered diluted honey, through Whatman no. 40 filter paper subsequent to its preparation for assay, we found that the riboflavin content was reduced 40% and the nicotinic acid values 25%. It is suggested that the large variation in the vitamin content of the honeys may be due in part to the variable amount of pollen present.

The values reported in this publication compare favorably with those of other workers. Pearson ('42) reports an average value of 30.3 μg. of pantothenic acid per gram of pollen and 511 μg. per gram (dry weight) of royal jelly. Cheldelin and Williams ('42) have analyzed royal jelly for the vitamins of the B complex. We find that our results agree very well with those of the Texas workers. Haydak and Palmer ('42b) report that chemical determinations did not show even traces of pyridoxine either in bee bread or royal jelly. The values

they obtained for the amount of nicotinic acid and pyridoxine found in honey are many times higher than our results. The discrepancy is so great that it is believed to be due to the methods of analyses employed. It is significant to note that Haydak and Palmer used chemical methods for both the nicotinic acid and pyridoxine determinations. It is probable that the treatment of the honey sample necessary to free the vitamins gave rise to interfering substances.

SUMMARY

Microchemical and microbiological determinations showed the presence in honey of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, and folic acid. The variation among samples was very large, due perhaps to the source of the honey and the number of pollen grains present. Comparison of new and aged honeys revealed a decrease in the pantothenic acid content of the latter. Pollen and royal jelly have also been assayed for these vitamins and have been found to be good sources of the B-complex. Royal jelly is very rich in biotin and pantothenic acid, which may be significant in the metabolism of the young bee.

LITERATURE CITED

ATKIN, L., A. S. SCHULTZ, W. L. WILLIAMS AND C. N. FREY 1943 Yeast microbiological method for the determination of pyridoxine. Ind. and Eng. Chem. (anal.), vol. 15, p. 141.

CHELDELIN, V. H., AND R. J. WILLIAMS 1942 The B vitamin content of foods. The University of Texas Publication No. 4237, p. 105.

GRIEBEL C. 1938 Vitamin C-enthaltende Honige. Ztschr. Untersuch. Lebensm., vol. 75, p. 417.

GRIEBEL, C., AND G. HESS 1939 Vitamin C-enthaltende Honige. Ztschr. Untersuch. Lebensm., vol. 78, p. 308.

———— 1940 Der C-Vitamingehalt des Blutennecktars bestimmter Labiaten. Ztschr. Untersuch. Lebensm., vol. 79, p. 168.

HAYDAK, M. H., AND L. S. PALMER 1942 a Vitamin content of honeys. J. Nutrition, vol. 23, p. 581.

———— 1942 b Royal jelly and bee bread as sources of vitamins B_1, B_2, B_6, C, nicotinic acid and pantothenic acid. J. Econ. Entom., vol. 35, p. 319.

HENNESSY, D. J. 1942 The determination of thiamin in cereal products. "Cereal Chemists Bull.", vol. 2.

SCHUETTE, H. A., AND D. J. HUENINK 1937 Mineral constituents of honey. II. Phosphorus, calcium, magnesium. Food Research, vol. 2, p. 529.

SCHUETTE, H. A., AND F. KAYE 1942 Master's Thesis. Univ. Wisconsin. Unpublished.

SCHUETTE, H. A., AND KATHORA REMY 1932 Degree of pigmentation and its probable relationship to the mineral constituents of honey. J. Am. Chem. Soc., vol. 54, p. 2909.

SCHUETTE, H. A., AND R. E. TRILLER 1938 Mineral constituents of honey. III. Sulfur and chlorine. Food Research, vol. 3, p. 543.

SCHUETTE, H. A., AND W. W. WOESSNER 1939 Mineral constituents of honey. IV. Sodium and potassium. Food Research, vol. 4, p. 349.

SNELL, E. E., R. E. EAKIN AND R. J. WILLIAMS 1940 A microbiological method for the determination of biotin. J. Am. Chem. Soc., vol. 62, p. 175.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. J. Ind. Eng. Chem. (anal.), vol. 11, p. 346.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 625.

STRONG, F. M., R. E. FEENEY AND A. EARLE 1941 Microbiological assay for pantothenic acid. J. Ind. Eng. Chem. (anal.), vol. 13, p. 566.

THE DESTRUCTIVE ACTION, IN VIVO, OF DILUTE ACIDS AND ACID DRINKS AND BEVERAGES ON THE RATS' MOLAR TEETH

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ONE PLATE (EIGHT FIGURES)

(Received for publication March 30, 1943)

A pronounced destruction of dentine and enamel of rats' molar teeth resulted from dilute acid solutions and certain acid drinks and beverages, consumed regularly in place of drinking water. In addition to effects observed both grossly and microscopically, differences in weights of comparable teeth of control and test rats gave a quantitative measure of actual amounts of dental tissue destroyed. The results point to the extreme sensitivity of rats' molar teeth, in vivo, to acid destruction, and suggest the possibility of a corrosive effect on human teeth resulting from excessive consumption of acid liquid-foods and beverages which may form a part of the human diet (Bridges and Mattice, '39). Acid foods such as ensilage, used extensively in animal nutrition, also may be suspected of having a corrosive action on the teeth.

In vitro studies have established acid solubility data for devitalized dentine and enamel (Rosebury, '38). Acid etching of enamel surfaces, in vitro, has been revealed by the reflecting microscope (Kanthak and Benedict, '32). Obviously results obtained in vitro do not constitute a measure of any acid-resistant properties of the live tooth, or of the acid-neutralizing effect of the saliva. Thus, although the destruction of tooth enamel by solutions of acidified candies was demonstrated in

vitro, it was suggested by West and Judy ('38) that the buffer action of the saliva would probably prevent any tissue destruction actually occurring in the mouth. Local oral effects resulting from consumption of certain foods have been studied largely as regards injury to the gingival tissues (Burwasser and Hill, '39; Pelzer, '40).

EXPERIMENTAL

Part I

In this experiment weanling rats, divided into litter-mate trios, were allowed as the fluid to drink (a) distilled water, (b) a dilute solution of hydrochloric acid (pH 1.5, 0.16%), and (c) a dilute solution of lactic acid (pH 2.4, 0.50%). All the rats were fed a stock diet having the following percentage composition: corn flour, 66; whole milk powder, 30; alfalfa meal, 3; and salt, 1. Food and drinking fluids were consumed ad libitum. At the end of specified periods (table 1) the rats were sacrificed. After autoclaving the heads, the molar teeth were dissected out, dried at 110°C., and weighed.

Both lactic acid and hydrochloric acid were very destructive to tooth enamel and dentine (table 1, figs. 2 and 3). As might be expected hydrochloric acid pH 1.5 was more destructive than lactic acid pH 2.4. Observed microscopically, dissolution was particularly severe on the lingual surface of the lower molars. The upper molars, apparently because of more limited contact with the drinking fluids, were less severely affected. As shown in figures 2 and 3, the crowns of the lower teeth were almost entirely destroyed by these acids.

On the occlusal surface the dentine of the rats' molars is exposed naturally. In several instances a hollowed-out or cupped effect on the occlusal surface was observed, due apparently to the greater acid solubility of the dentine than of the enamel. In general, however, at these acid levels the enamel appeared to be affected at about the same rate as the dentine.

Differences in weights of individual molar teeth of control and test rats (table 1) indicate quantitatively actual weights of dental tissue destroyed. These weight figures, obtained

TABLE 1

Effect of dilute hydrochloric acid and dilute lactic acid as drinking fluids on rats' molar teeth.

DRINKING FLUID [1]	DAYS ON EXPERIMENT	INITIAL BODY WT.	FINAL BODY WT.	WEIGHT OF MOLAR TEETH						LOSS IN WEIGHT	
				Right-lower			Right-upper			Right lower	Right upper
				1st	2nd	3rd	1st	2nd	3rd		
		gm.	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	%	%
Water	42	44.1	114.5	10.6	6.5	4.3	12.1	6.9	3.0	...	..
Dilute HCl	42	33.7	96.0	8.3	5.4	2.6	10.9	6.4	2.7	23.8	9.1
Dilute lactic	42	36.2	109.5	9.1	5.7	3.0	11.5	6.5	2.8	16.8	5.5
Water	49	36.6	143.0	12.5	8.8	5.5	13.5	8.4	4.3	...	..
Dilute HCl	49	32.3	96.0	9.9	6.3	3.4	10.9	6.3	3.0	26.9	22.9
Dilute lactic	49	35.4	139.5	10.8	6.8	3.9	12.3	7.0	3.3	19.7	11.8
Water	56	27.9	107.5	11.5	7.9	5.1	12.5	7.9	3.8	...	..
Dilute HCl	56	24.8	109.5	7.3	4.3	2.5	8.3	4.7	2.2	42.4	37.2
Dilute lactic	56	27.2	116.5	8.7	5.7	3.4	10.2	5.8	2.4	27.3	23.9
Water	56	26.5	143.0	13.9	9.1	5.0	13.8	8.2	3.3	...	..
Dilute HCl	56	24.4	155.0	6.4	4.2	2.9	10.6	6.0	3.0	51.7	22.5
Dilute lactic	56	31.0	87.5	7.3	5.6	3.1	13.2	7.1	3.6	42.9	5.5
Water	56	36.0	130.0	12.9	8.6	5.0	13.8	7.1	3.3	...	..
Dilute HCl	56	25.2	136.4	8.8	5.2	2.7	12.5	5.8	2.5	36.9	14.0
Dilute lactic	56	23.4	162.5	8.0	4.7	3.1	12.4	7.5	3.3	40.4	4.1
Water	63	30.0	138.2	13.1	9.0	5.6	13.7	8.5	3.2	...	..
Dilute HCl	63	35.0	100.0	6.8	4.5	2.4	11.1	5.3	2.5	50.5	25.6
Dilute lactic	63	25.3	153.5	8.2	5.0	2.6	11.8	7.2	2.8	43.0	14.1
Water	63	23.4	125.4	12.0	8.2	4.8	12.1	7.5	3.2	...	..
Dilute HCl	63	29.2	130.0	9.1	6.0	3.2	10.9	5.8	2.7	26.8	14.9
Dilute lactic	63	23.3	108.0	11.5	6.9	3.5	12.0	7.4	2.7	12.4	3.1
Water	70	19.0	103.5	11.0	7.4	4.0	11.9	7.0	3.0	...	..
Dilute HCl	70	22.9	151.0	9.4	5.6	3.0	11.6	6.2	2.6	19.6	6.8
Dilute lactic	70	33.2	132.0	9.9	6.3	3.5	11.6	6.8	2.9	12.1	2.7
Water	70	24.6	133.0	12.3	8.0	4.8	12.8	7.9	3.8	...	..
Dilute HCl	70	25.5	152.0	8.6	5.1	2.2	10.5	5.8	2.5	36.7	23.3
Dilute lactic	70	21.8	118.5	10.9	6.9	4.0	13.0	7.2	3.6	13.1	2.9
Water	70	24.8	125.0	12.3	8.1	4.8	13.7	8.5	3.7	...	..
Dilute HCl	70	24.0	130.0	8.3	4.9	2.8	10.5	6.0	3.0	36.5	24.7
Dilute lactic	70	24.9	151.0	10.4	6.3	3.2	12.8	7.2	3.6	21.0	8.9
Water	70	23.2	116.5	13.1	8.5	4.8	13.4	7.8	3.5	...	..
Dilute HCl	70	21.6	128.0	10.2	6.5	2.7	12.4	6.7	2.8	26.5	11.3
Dilute lactic	70	22.2	121.5	10.5	6.7	3.3	12.9	7.6	3.5	22.3	2.8
Averages for each drinking fluid											
Water	..	28.8	125.4	12.3	8.2	4.9	13.0	7.8	3.5	...	..
Dilute HCl	..	27.1	125.5	8.5	5.3	2.8	10.9	5.9	2.7	34.6	19.8
Dilute lactic	..	27.6	127.3	9.6	6.1	3.3	12.2	7.0	3.1	25.2	8.2

[1] Hydrochloric acid 0.16%, pH 1.5; lactic acid 0.50%, pH 2.4; distilled water pH 6.5–7.0.

under the condition of controlled fluid intakes, afford a quantitative means of comparing one acid fluid with another (table 2, experiment 3). Any suggestion of a difference related to the length of the exposure, among the rats shown in table 1, would have to be confirmed by equalizing the quantities of liquid drunk. Variations in results obviously may be brought about by differences in the rats' drinking habits and possibly by differences in individual susceptibilities.

TABLE 2

Weight of rats' molar teeth following consumption of a ginger ale, a cola drink, a grapefruit juice, and a cranberry juice cocktail as the only drinking fluids.

	WATER		GINGER ALE		COLA DRINK		GRAPE-FRUIT JUICE		CRANBERRY JUICE COCKTAIL	
Drinking fluid reaction (pH)	6.5–6.6		2.8–3.2		2.6–2.7		3.2–3.5		2.5–2.6	
Drinking fluid total acidity (normality)	..		.06		.04		.22		.14	
Experiment number	2	3	2	3	2	3	2	3	2	3
No. of rats averaged	10	12	10	12	10	12	10	12	8	12
Days on experiment	82	84	82	82	82	82	82	85	80	82
Initial body weight (gm.)	33	42	27	41	33	42	30	40	30	44
Final body weight (gm.)	198	182	207	183	194	183	201	196	179	180
Average daily drink (ml.)	..	20.1	..	23.0	..	22.9	..	22.5	..	23.0
Average weight of molar teeth										
Right lower										
1st molars (mg.)	15.6	15.0	14.8	13.7	14.2	13.9	12.8	13.6	9.1	10.4
2nd molars (mg.)	9.9	9.9	9.2	9.0	8.9	9.0	7.9	9.1	5.8	7.2
3rd molars (mg.)	6.2	6.6	5.5	5.9	5.3	5.7	4.4	5.3	2.9	4.2
Right upper										
1st molars (mg.)	16.2	15.1	16.2	15.4	15.4	15.5	16.0	16.3	13.3	14.8
2nd molars (mg.)	9.7	9.4	9.6	9.5	9.3	9.4	9.7	10.3	7.5	9.3
3rd molars (mg.)	5.2	5.3	5.1	5.5	5.1	5.4	5.2	5.7	2.9	4.8

In addition to the results for lactic acid (pH 2.4) and hydrochloric acid (pH 1.5) it has been observed that citric acid (pH 2.5) (fig. 4) and lactic acid (pH 3.0 and 4.0) will corrode rats' molar teeth. The upper molars were not changed significantly.

Part II

The above study was followed by experiments 2 and 3 (table 2) in which the following liquids were drunk by young rats in place of water: (a) ginger ale pH 2.8–3.2; (b) a cola drink pH 2.6–2.7; (c) grapefruit juice pH 3.2–3.5 and (d) a cranberry juice cocktail pH 2.6–2.7. These drinks and beverages were obtained from local food stores. The ration was a commercial one.[1] In experiment 2 eating and drinking were allowed ad libitum, and in experiment 3, with the exception of water, fluid consumption was equalized. Fresh allotments of drinking fluid were given each day. These beverages did not change in pH after standing 24 hours at room temperature.

The weights of the molar teeth of these rats are shown in table 2. Differences between control and test rats are most pronounced in the lower molar teeth. These weight differences minimize the corrosive action because a large portion of the weight of the tooth consists of the unexposed root and it would appear also that in general the buccal surfaces of the teeth have relatively little contact with the drinking fluids. During the drinking process the lower molars seemingly are in contact with the drinking fluids more than the uppers. This is shown by the weight data and by the appearance of the teeth. As observed microscopically, all the rats given acid fluids showed an unmistakable corrosion on the lower molars in particular. The photographs (figs. 5, 6, 7 and 8) also show this effect. In the rats given cranberry juice cocktail the crown of the lower molars was frequently reduced to the gingival line. The most serious action resulted from this fluid. In experiment 3 where the fluid intakes were controlled, the other three fluids were similar in their corrosive action. There was some tendency for the upper molars of control rats to weigh slightly less than the upper molars of test rats. This may be explained possibly by a greater loss from normal occlusal wear in these rats than in the test rats. As a result of the acid fluids the lower molars of test rats frequently showed a more or less

[1] Stock rat ration, a product of the Maritime Milling Co., Buffalo, New York.

smooth diagonal surface which presumably lessened the normal wear on the opposing upper molars.

An estimate of the pH of the rats' oral cavities was obtained by means of a nasal electrode placed in the mouths of rats while they were under the influence of a mild ether anesthetic. These readings on eight rats equaled pH 8.2, 8.2, 8.0, 7.7, 8.0, 7.8, 7.9, and 8.0. Several 1 ml. volumes of washings from rats' oral cavities averaged pH 7.8 to pH 8.0. The possibility that a reduced buffering action of rat saliva contributed to these acid effects seems negligible.

DISCUSSION

A current conception of the etiology of dental caries is the decalcification of the dental tissues by acids of bacterial origin particularly lactic acid (Miller, 1890). The acid resistant properties of teeth would appear to play an important rôle in the prognosis of the disease. These results pertaining to rats' teeth in vivo suggest the possibility that oral tooth surfaces may be affected by acid drinking fluids passing through the oral cavity. Any association of these effects with the initiation of dental caries however, remains to be determined. The experimental procedure permits the observation in vivo of the susceptibility of dental tissues to various acids of dietary or bacterial origin, and has the additional advantage of furnishing quantitative data relative to the actual quantities of dental tissue destroyed.

LITERATURE CITED

BRIDGES, M. A., AND MARJORIE A. MATTICE 1939 Over two thousand estimations of the pH of representative foods. Am. J. Digest. Diseases, vol. 6, p. 440.

BURWASSER, P., AND T. J. HILL 1939 Effect of hard and soft diets on gingival tissues of dogs. J. Dent. Res., vol. 18, p. 389.

KANTHAK, F. F., AND H. C. BENEDICT 1932 Effect of dilute acids on the enamel surface as disclosed by the reflecting microscope. Dent. Cosmos, vol. 74, p. 439.

MILLER, W. D. 1890 The micro-organisms of the human mouth. S. S. White, Dent. Mfg. Co., Phila.

PELZER, R. H. 1940 A study of the local oral effect of diet on the periodontal tissues and gingival capillary structures. J. Am. Dent. Assoc., vol. 27, p. 13.

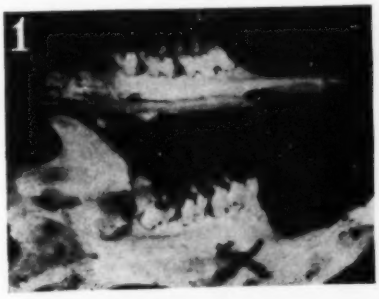
ROSEBURY, T. 1938 The problem of dental caries. In Dental Science and Dental Art. S. M. Gorden ed. Lea & Febiger, Phila., chapter 8.

WEST, E. S., AND F. R. JUDY 1938 Destruction of tooth enamel by acidified candies. J. Dent. Res., vol. 17, p. 499.

PLATE 1

EXPLANATION OF FIGURES

Enlarged photographs showing corrosive effect of dilute acids and acid drinks and beverages on the rats' molar teeth: (1) control, distilled water pH 6.5–7.0; (2) hydrochloric acid pH 1.5; (3) lactic acid pH 2.4; (4) citric acid pH 2.5; (5) a cola drink pH 2.6–2.7; (6) a ginger ale pH 3.2–3.4; (7) grapefruit juice pH 3.2–3.5; (8) a cranberry juice cocktail pH 2.5–2.6.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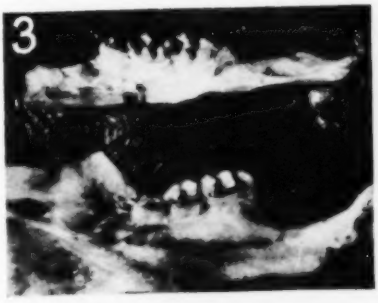
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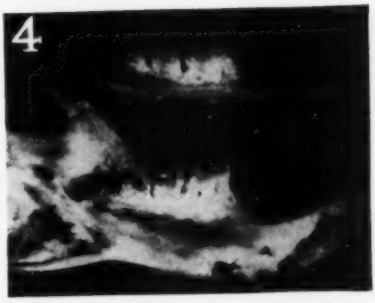
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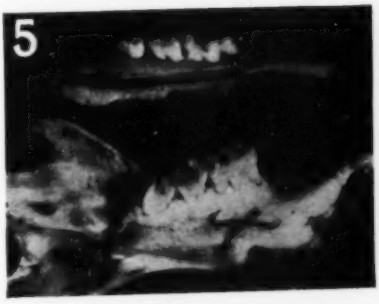
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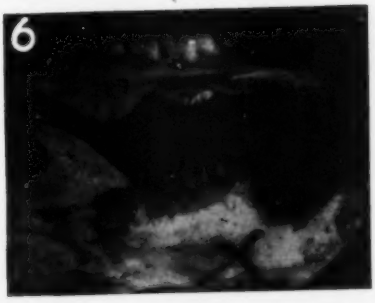
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THE INFLUENCE OF THE THIAMINE INTAKE OF THE PIG ON THE THIAMINE CONTENT OF PORK WITH OBSERVATIONS ON THE RIBOFLAVIN CONTENT OF PORK [1]

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(Received for publication March 13, 1943)

It is well-established that pork is an outstanding source of thiamine. A survey of the literature reveals, however, that there is considerable variation in the thiamine values which have been reported for pork. While some of these differences might be attributed to methods of determination, and to normal or expected variation, it is logical to suppose that certain physiological factors might also be responsible, at least in part, for some of the diversity among reported thiamine values for pork.

Among such factors are thiamine saturation of tissues (Schultz, Light and Frey, '38; Schultz, Light, Cracas and Atkin, '39), the relationship of thiamine to the glycogen cycle in muscle (Pyke, '40), and the thiamine sparing action of fats (Ellis and Madsen, '42).

It is a generally accepted fact that increased intake of carbohydrate increases the thiamine requirement; Waisman and Elvehjem ('41) have commented on this fact in connection with swine which are fed rations characteristically rich in

[1] Authorized for publication on March 11, 1943, as paper no. 1171 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Part of the expenses of this investigation were defrayed by a fellowship grant from Swift and Company, Chicago, Illinois.

carbohydrate. It is possible that the characteristically high thiamine content of pork is the result of demands on the muscle tissue for thiamine to aid in the metabolism of carbohydrate, including its conversion to fat.

A further possibility is that the thiamine content of pork might be directly influenced by the thiamine intake of the pig. Some data bearing on this point have been presented by Pyke ('40) who reported thiamine values for the longissimus dorsi and psoas (back and loin) muscles from groups of two, five and four pigs fed, respectively, on swill, commercial meal (190 I. U. thiamine per 100 gm.), and the same commercial meal supplemented with crystalline thiamine to raise its thiamine content to 850 I. U. per 100 gm. No assay values are reported for the swill, but it is stated that it almost certainly contained less vitamin B_1 than the cereal meal. The amount of thiamine in the psoas muscle was shown to be consistently greater than that present in the longissimus dorsi. The thiamine values for these muscles were very similar for the pigs fed the plain meal and for those fed the meal supplemented with thiamine, but the values were considerably higher, in each instance, than those for the two swill-fed pigs. Pyke concludes that a normal pig ration supplies all the vitamin B_1 that the pig is capable of storing, and that the reduction in muscle thiamine in the swill-fed pigs is due to a deficiency of thiamine in the diet.

The present investigation was undertaken to obtain further data dealing with the relationship between the thiamine intake of the pig and the thiamine content of the pork. Although the study was primarily concerned with thiamine, riboflavin values were also determined owing to the paucity of riboflavin values reported in the literature.

EXPERIMENTAL

The feeding experiment was conducted during the period July to November, 1942. Twenty-seven pigs were assigned to three lots of nine pigs each, with the distribution between lots being made as nearly equal as was possible with respect

to breed, sex and initial live weight (table 2). Throughout most of the feeding period the pigs were kept on the ground in half-acre outdoor feed lots which were devoid of vegetation. Suitable shelter against the sun and inclement weather was provided, and in the latter part of the experiment, in colder weather, the pigs were housed indoors.

The pigs were trough-fed in groups, twice daily. Data on feed consumption were recorded and no more feed was offered than could be readily consumed. A suitable amount of water was added to the feed at feeding time.

TABLE 1

Composition of rations.

	INGREDIENTS		
	Lot 1	Lot 2	Lot 3
	%	%	%
Yellow corn	57.0	49.0	53.5
Tankage (60% protein)	5.5	5.5	11.0
Soybean oil meal	10.0	10.0	—
Linseed oil meal	10.0	10.0	—
Dehydrated alfalfa leaf meal	5.0	5.0	10.0
Whole wheat	—	10.0	15.0
Peanut skins	10.0	10.0	—
Corn gluten meal	—	—	10.0
Dried brewers' yeast	2.0	—	—
Salt	0.48	0.48	0.48
Manganese	0.02	0.02	0.02
	100.00	100.00	100.00
	ANALYSIS		
Protein (%)	19.95	19.35	19.78
Thiamine (μg. per lb.)	5761	3447	1315
Riboflavin (μg. per lb.)	1801	1669	2468

The composition of the rations is given in table 1. The protein content of the rations is considered adequate for pigs weighing 40 to 50 pounds, but it is higher than that required for pigs weighing from 75 to 200 pounds. Rations 1, 2 and 3, fed to lots 1, 2 and 3, respectively, were designed primarily to furnish three levels of thiamine, the respective amounts being 5761, 3447 and 1315 μg. of thiamine per pound of feed. Ex-

traordinary sources of thiamine were red peanut skins, used in rations 1 and 2, and brewers' yeast, used in ration 1. Some difficulty was experienced in obtaining brewers' yeast which contained sufficient thiamine to provide the desired amount in ration 1. Consequently, the required additions of pure thiamine (in solution) were mixed with this ration just prior to feeding.

Three pigs, one from each lot, were slaughtered on the 100th day of the feeding experiment. Thereafter, usually at weekly intervals, one pig from each lot was slaughtered. Thus the length of the feeding period varied from 99 to 140 days, respectively, for the first and last pigs slaughtered from each lot. After slaughter the carcasses were chilled for at least 24 hours at 37°F. before samples of the pork were taken for analysis. Samples were taken from the ham end of the loin, the center of the loin, the shoulder and the liver. Adhering fat was removed from the samples which were prepared for analysis by grinding in a food chopper. Thus, all analyses represent lean tissue.

Whenever possible, samples for the determination of dry matter, crude protein and thiamine were weighed immediately after the sample had been ground and thoroughly mixed. Otherwise samples were frozen immediately in the freezing compartment of an electric refrigerator and kept in the frozen state until they were analyzed. All of the riboflavin determinations were made on samples which had been kept in the frozen state.

Thiamine was determined by the thiochrome method, using the procedure recommended for cereal products (Hennessy, '42), and riboflavin was determined by the fluorometric method of Conner and Straub ('41). These methods were modified only in the manner of preparation of the extracts; a weighed amount of the previously ground fresh or frozen sample was mixed in a Waring blender with a suitable volume of extractant, from which mixture aliquots were pipetted into volumetric flasks.

TABLE 2

Weights, feed consumption and economy of gains of pigs with different levels of thiamine intake.

LOT NO.	THIAMINE CONTENT OF FEED	PIG NO.	BREED	SEX	AGE AT START OF EXPERIMENT	NUMBER OF DAYS ON FEED	INITIAL LIVE WEIGHT	FINAL LIVE WEIGHT	TOTAL GAIN IN LIVE WEIGHT	AVERAGE DAILY GAIN	AVERAGE DAILY FEED	FEED REQUIRED PER 100 LBS. GAIN IN LIVE WEIGHT
	µg/lbs.				*days*		*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	5761	988	Chester White	Barrow	88	99	55	209	154	1.56	4.7	301
		990	Poland China	Barrow	74	105	49	207	158	1.50	4.8	319
		996	Chester White	Barrow	88	111	56	205	149	1.34	5.0	372
		995	Duroc Jersey	Gilt	74	112	44	200	156	1.39	5.0	361
		65	Duroc Jersey	Gilt	74	119	50	205	155	1.30	5.2	395
		70	Berkshire	Gilt	77	127	44	205	161	1.27	5.3	329
		74	Chester White	Gilt	88	133	34	205	171	1.29	5.4	422
		916	Poland China	Barrow	74	140	49	197	148	1.06	5.5	522
				Average	80	118	48	204	157	1.34	5.1	378
2	3447	992	Chester White	Gilt	88	99	61	226	165	1.67	4.9	297
		991	Chester White	Barrow	88	105	56	211	155	1.48	5.0	340
		998	Chester White	Barrow	88	111	49	200	151	1.36	5.2	382
		997	Duroc Jersey	Gilt	74	112	50	210	160	1.43	5.2	365
		66	Duroc Jersey	Barrow	74	119	47	220	173	1.45	5.2	278
		71	Poland China	Barrow	74	127	41	222	175	1.38	5.4	390
		75	Berkshire	Gilt	82	133	35	202	167	1.26	5.5	435
		917	Berkshire	Barrow	102	140	31	167	136	0.91	5.4	560
				Average	84	118	46	207	160	1.37	5.2	381
3	1315	994	Chester White	Barrow	88	99	52	212	160	1.62	5.0	308
		993	Duroc Jersey	Gilt	74	105	50	200	150	1.43	5.1	354
		999	Duroc Jersey	Barrow	74	111	45	200	155	1.40	5.2	375
		1000	Duroc Jersey	Gilt	74	112	47	218	171	1.53	5.3	345
		68	Chester White	Gilt	88	119	47	210	163	1.37	5.3	260
		73	Berkshire	Barrow	82	127	45	185	140	1.10	5.4	492
		915	Chester White	Gilt	88	133	49	198	149	1.12	5.5	494
		918	Poland China	Barrow	74	140	40	210	170	1.21	5.7	466
				Average	80	118	47	204	157	1.35	5.3	387

EXPERIMENTAL RESULTS AND DISCUSSION

Data pertaining to the body gains and feed consumption of the pigs are given in table 2. It is shown that, on the average, in spite of individual differences, the three lots of pigs were quite similar with respect to growth and feed requirements. It is apparent that the ration for lot 3, which furnished the least amount of thiamine was fully adequate to fulfill the pigs' requirements for this factor. In this case the average daily feed intake of 5.3 lbs. supplied 7 mg. of thiamine which is in

TABLE 3

Average proximate analyses of pork.

LOT NO.		1	2	3
NUMBER OF PIGS		8	8	8
THIAMINE CONTENT OF FEED (μG./LB.)		5761	3447	1315
SOURCE OF SAMPLE		%	%	%
Shoulder	Dry matter	27.0	28.5	28.2
	Protein in fresh pork	19.2	19.2	19.5
Center loin	Dry matter	27.8	27.6	29.2
	Protein in fresh pork	21.7	21.8	21.8
Ham end of loin	Dry matter	26.9	28.0	28.4
	Protein in fresh pork	21.4	21.8	21.2
Liver	Dry matter	30.5	31.0	30.5
	Protein in fresh liver	23.1	22.5	22.2

excess of the suggested daily requirement of 1 to 3 mg. per 100 lbs. of body weight. The results show, further, that there was no apparent advantage derived from the additional thiamine provided in the rations of lots 1 and 2 so far as growth and feed utilization are concerned.

The average proximate analyses of the pork are given in table 3. Again, in spite of individual differences, there is general uniformity of the results for the pigs of the three lots.

Data for the thiamine content of the pork are given in table 4. Thiamine values for the muscle tissues directly reflect

The thiamine content of pork as influenced by the thiamine content of the feed (thiamine expressed as μg./gm. of fresh tissue, dry matter and protein).

The thiamine content of pork as influenced by the thiamine content of the feed (thiamine expressed as μg./gm. of fresh tissue, dry matter and protein).

PIG NO.	SHOULDER			CENTER LOIN			HAM END OF LOIN			LIVER		
	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein	Fresh	Dry matter	Protein
Lot 1 — 5761 μg. of thiamine per pound of feed												
988	16.0	51.7	87.7	20.1	61.8	99.0	22.3	80.2	106.8	5.9	19.3	24.2
990	13.1	44.2	65.7	18.3	69.9	81.3	17.8	65.5	80.9	2.7	9.5	12.2
996	19.4	76.6	95.2	25.1	86.1	110.9	26.3	93.5	120.9	7.3	24.5	31.8
995	17.9	63.8	96.1	25.3	87.8	112.3	24.4	93.9	120.5	6.2	20.7	26.7
65	14.7	59.2	80.3	23.3	86.5	109.6	22.6	87.8	110.2	6.2	19.9	27.5
70	17.2	69.5	85.4	23.8	89.5	106.1	21.8	81.7	97.7	3.6	11.9	16.2
74	19.2	71.2	102.1	22.8	79.6	105.4	25.8	90.9	123.6	5.6	17.3	24.5
916	21.1	82.5	109.2	27.8	106.9	125.3	29.9	117.0	132.5	5.2	16.6	21.4
Average	17.3	64.8	90.2	23.1	83.5	106.2	23.9	88.8	111.6	5.3	17.5	23.1
Standard error	0.9	4.5	4.8	1.0	4.8	4.4	1.3	5.2	5.6	0.5	1.7	4.8
Lot 2 — 3447 μg. of thiamine per pound of feed												
992	13.1	41.8	69.4	19.9	69.6	90.5	18.6	63.2	85.3	4.8	15.5	20.1
991	16.8	57.7	86.7	23.4	80.9	106.7	24.2	86.8	108.8	4.8	15.7	20.4
998	15.5	51.1	78.5	18.3	65.3	82.2	19.6	67.1	85.4	4.4	15.3	21.9
997	16.9	57.5	87.5	20.7	77.8	92.0	19.4	70.3	93.5	5.1	15.9	21.5
66	12.6	49.6	67.0	20.1	77.1	94.6	19.3	76.0	91.3	5.5	18.0	23.0
71	14.0	46.2	75.4	18.8	65.8	90.9	19.2	64.2	89.8	4.2	14.2	18.9
75	13.8	52.7	69.0	19.0	69.0	84.0	19.9	72.9	88.2	3.9	11.8	18.1
917	17.8	68.3	92.5	20.1	76.7	93.8	20.5	75.8	95.3	3.0	9.3	14.2
Average	15.1	53.1	78.3	20.0	72.8	91.8	20.1	72.0	92.2	4.5	14.5	19.8
Standard error	0.7	2.9	2.9	0.6	2.1	2.6	0.6	2.7	2.7	0.3	1.0	1.0
Lot 3 — 1315 μg. of thiamine per pound of feed												
994	6.5	20.1	35.6	9.2	28.7	44.3	10.4	37.3	52.0	4.2	13.9	18.6
993	5.9	23.5	31.0	7.3	24.5	32.7	11.1	38.5	52.1	3.8	11.9	15.1
999	10.1	37.4	49.0	9.6	30.9	43.1	10.0	33.1	48.3	3.6	10.7	16.6
1000	7.7	25.1	41.3	9.2	31.5	45.3	8.8	28.6	44.3	3.5	11.5	15.2
68	7.1	28.0	33.9	10.5	40.4	45.7	9.5	36.4	42.2	2.5	8.2	12.1
73	9.7	35.2	46.6	10.4	36.7	45.8	12.1	43.4	52.6	1.9	6.4	8.2
915	7.5	26.4	39.1	9.7	35.9	44.0	9.5	33.8	43.3	2.4	8.2	10.5
918	8.9	30.6	47.3	10.3	33.9	49.9	11.2	41.3	55.1	4.5	15.5	24.1
Average	7.9	28.3	40.5	9.5	32.8	43.9	10.3	36.6	48.7	3.3	10.8	15.1
Standard error	0.5	2.1	3.1	0.4	1.8	1.8	0.4	1.7	1.7	0.3	1.1	1.8

the thiamine intakes of the pigs, and the data show that much higher amounts of thiamine were present in the muscle tissues of the pigs of lots 1 and 2 than was the case for similar tissues of the pigs of lot 3. The thiamine content of the pork from the pigs of lot 2, which received 3447 μg. of thiamine per pound of feed, is approximately twice that of the pigs of lot 3, which received 1315 μg. of thiamine per pound of feed. In the case of lot 1, which received 5761 μg. of thiamine per pound of feed, the average thiamine values for the pork are higher, in all instances, than those for the pork from the next lower thiamine lot (no. 2), but, although the trend of the values seems to be established, the differences are not statistically significant in all cases. The above relationships are true whether or not the comparisons are made on the basis of the thiamine content of the fresh pork, dry matter, or total protein. Although the thiamine values for the pork from lot 1 are higher than those for lot 2, the differences are not commensurate with the differences in the thiamine intake of these two lots. A more direct relationship exists in the case of lots 2 and 3 where an increase of 2.6 times in thiamine intake resulted in approximately doubling the thiamine content of the pork.

It is interesting to note that there seems to be a preferential attraction of pork muscle for thiamine as compared to liver. Furthermore, liver seems to be much less responsive to the effect of added dietary thiamine. It will be noted, in comparing lots 2 and 3, that the increase in thiamine content of liver, as the result of thiamine feeding, was of the order of 40 to 70%, as contrasted with about 100% for muscle. Pork muscle is unique in its ability to absorb and retain thiamine and appears to be the principal repository of this vitamin.

Our findings with respect to the increased thiamine content of pork muscle as the result of feeding are somewhat similar to those reported by Pyke ('40), but they differ in several respects the first of which is the magnitude of the thiamine values for pork from pigs with more or less comparable intakes of thiamine.

The limited data of Pyke show a range of from 12 to 14.55 μg. of thiamine per gram of loin on feeds which ranged from 2586 to 11,567 μg. of thiamine per pound of feed. Our thiamine values are somewhat higher, ranging from 18.3 to 27.8 μg. per gram of loin from pigs which received 3447 and 5761 μg. of thiamine per pound of feed. In spite of the difference in the magnitude of the thiamine values, our observations and those of Pyke show that different muscle tissues from the same pig may contain different amounts of thiamine. In our studies the loin values exceeded those of the shoulder by 20 to 25%. No data are available showing whether there may be a variation in the thiamine content of different parts of the same muscle.

Pyke's results show little, if any, increase in the thiamine values for pork when the level of thiamine feeding was increased from 2586 to 11,567 μg. per pound of feed, although the values at these levels were approximately double those for swill-fed hogs. Our values for pigs fed 3447 μg. of thiamine per pound of feed are approximately 30% higher than those reported by Pyke at the highest level of feeding (11,567 μg. per pound of feed), and in each instance our values show some increase as a result of a higher level of thiamine intake.

There does not appear to be any satisfactory explanation for the above points of difference. Feed consumption might be a factor influencing thiamine storage. Pyke does not report the feed intakes, but in our case it was practically the same for the three lots of pigs. Our results do not indicate any marked influence of breed on the thiamine content of the pork. However, Pyke's data indicate that breed might be a factor, and it is possible that the Wessex Saddleback pig might differ in this respect from the breeds used in our investigation. We feel, however, that the species rather than the breed, in the case of swine, characterizes the great ability of the pig to accumulate thiamine in its muscle tissue.

Our results, which show that the thiamine content of pork may be influenced by the thiamine intake of the pig, and that different pork cuts may vary in thiamine content, are of

particular interest in helping to explain some of the wide variations in thiamine values for pork which have been reported in the literature. Our work does not fully delineate the limits within which the thiamine content of pork may vary, but some indication of the upper limit is indicated by the results obtained by feeding 5761 μg. of thiamine per pound of feed to lot 1, and by further data given in table 5.

TABLE 5

Thiamine and riboflavin values for pork from pigs receiving 50 mg. of additional thiamine hydrochloride daily for a period of 22 days

PIG. NO.		69 [1]	72 [2]	69	72
SOURCE OF SAMPLE	BASIS	THIAMINE		RIBOFLAVIN	
		μg./gm.	μg./gm.	μg./gm.	μg./gm.
Shoulder	Fresh	15.5	14.1	3.5	3.7
	Dry matter	59.8	50.1	13.5	13.1
	Protein	79.2	71.6	17.9	18.7
Center loin	Fresh	22.2	18.6	1.8	2.4
	Dry matter	89.2	66.2	7.4	8.4
	Protein	101.5	86.3	8.4	10.9
Ham end of loin	Fresh	21.8	18.1	2.9	3.3
	Dry matter	85.7	66.3	11.3	12.0
	Protein	102.3	84.7	13.4	15.3
Liver	Fresh	5.6	5.8	51.7	46.9
	Dry matter	19.0	17.0	175.3	137.5
	Protein	26.0	23.8	239.8	192.4

[1] Pig no. 69 received feed containing 5761 μg. thiamine per pound.
[2] Pig no. 72 received feed containing 1315 μg. thiamine per pound.

One pig from the original lot 2 was affected with piles and was removed from the experiment. Its mates in lots 1 and 3, nos. 69 and 72, were continued on the experiment and received the same feeds as the other members of lots 1 and 3, with the addition, during the last 22 days of the experiment, of a daily supplement of 50 mg. of pure thiamine. The results, as given in table 5, show that there was no accumulation of this additional thiamine in the muscle tissue of the pig (no. 69) on the high thiamine (5761 μg. per pound) feed; however,

a very appreciable storage of thiamine did occur in the case of pig no. 72, which had received only 1315 μg. of thiamine per pound of feed. The thiamine values for the pork of this pig are of the same order as those for the pork of the pigs of lots 1 and 2, and indicate that thiamine may be very rapidly accumulated in pork muscle tissue under conditions of high thiamine intake.

Schultz, Light, Cracas and Atkin ('39) have pointed out that storage of thiamine in the accepted sense, does not take place in the body of the rat. We feel that probably this is also true in the case of the pig. This viewpoint may involve a very strict differentiation between storage and accumulation. Since our limited data indicate that thiamine may be accumulated rapidly in pork muscle, under conditions of high thiamine intake, it is felt that its disappearance from the tissue may be equally rapid. Thus, thiamine accumulation in the tissue, even to the point of saturation, may not constitute true storage.

The data presented in this paper do not give a complete picture with respect to the average values and the lower limit of the thiamine values which may apply to pork in general and which may be determined by the thiamine intake of the average hog. Limited data are available for the thiamine content of hog feeds. It may be assumed, however, that many hog rations, especially those in which corn predominates heavily, are not rich in thiamine. On the other hand, hogs fed peanuts, peanut by-products, certain oil meals, yeast and other thiamine-rich feed ingredients ingest considerable amounts of thiamine. With the lack of any specific data to prove the point, we feel that 1315 μg. of thiamine per pound of feed, the amount present in the ration of lot 3, represents a thiamine intake for hogs which is probably somewhat higher than the average. Thus, the thiamine values for the pork of lot 3 are probably at least not lower than the average.

Average riboflavin values for pork from lots 1, 2 and 3 are given in table 6. No attempt was made to control the riboflavin content of the feeds. Consequently, the variations in this vitamin in the feeds, were not comparable to those of

thiamine. It will be noted that the riboflavin content of pork muscle is much lower than that of thiamine while pork liver is rich in riboflavin but relatively low in thiamine.

TABLE 6

The riboflavin content of pork.

LOT NO.		1		2		3	
NUMBER OF PIGS		8		8		8	
RIBOFLAVIN CONTENT OF FEED (μG./LB.)		1801		1669		2468	
SOURCE OF SAMPLE	BASIS	AVERAGE RIBOFLAVIN CONTENT OF PORK (EXPRESSED AS μG./GM. OF FRESH TISSUE. DRY MATTER AND PROTEIN)					
		μg./gm.	*Standard error*	*μg./gm.*	*Standard error*	*μg./gm.*	*Standard error*
Shoulder	Fresh	3.5	0.1	3.3	0.1	3.2	0.1
	Dry matter	13.0	0.7	11.5	0.5	11.5	0.9
	Protein	18.2	0.6	17.0	0.7	16.4	0.7
Center loin	Fresh	2.2	0.1	2.3	0.1	2.2	0.1
	Dry matter	7.9	0.4	8.1	0.4	7.5	0.4
	Protein	10.1	0.4	10.3	0.6	10.1	0.3
Ham end of loin	Fresh	3.2	0.2	3.0	0.2	3.0	0.2
	Dry matter	11.8	0.7	10.6	0.5	10.7	0.6
	Protein	14.9	1.0	13.6	0.6	14.3	0.7
Liver	Fresh	43.8	0.9	41.8	0.8	40.5	1.0
	Dry matter	143.7	3.0	134.9	2.3	132.7	2.7
	Protein	189.6	2.9	185.9	3.0	182.5	3.5

SUMMARY

Three lots of pigs were fed rations containing 5716, 3447 and 1318 μg. of thiamine per pound of feed, respectively. The average feed consumption was approximately the same for the three lots of pigs. Thus, the average daily thiamine intakes were approximately 29, 17 and 7 mg., respectively.

Results with respect to growth and feed consumption were similar for the three lots of pigs in spite of the differences in thiamine intake.

Thiamine values were determined on pork samples taken from the shoulder, the middle of the loin, the ham end of the

loin and the liver. An increase of approximately 100% in the thiamine content of pork muscle resulted when the thiamine intake was increased from 1318 to 3447 μg. per pound of feed. A similar increase of the order of 15 to 20% resulted from an increase in the thiamine content of the feed from 3447 to 5761 μg. per pound.

The average thiamine values, on the fresh basis, for shoulder, center loin and ham end of loin, at the lower level of thiamine intake, were 7.9, 9.5 and 10.3 μg. per gram, respectively. At the high level of thiamine intake the corresponding values were 17.3, 23.1 and 23.9 μg. per gram.

The thiamine content of pork loin was found to be consistently higher than that of pork shoulder. Pork liver, as compared to muscle, was found to be relatively low in thiamine content. There was some increase in the thiamine content of the liver as the result of feeding more thiamine, but the main depository of thiamine in the pig is the muscle tissue.

Limited observations with but two pigs seem to indicate that pork muscle tissues have nearly reached the saturation point, so far as thiamine storage is concerned, when the thiamine content of the ration approximates 5800 μg. per pound. As the result of these preliminary observations it is concluded that thiamine accumulation in pork muscle is relatively rapid. These experiments are being continued.

Riboflavin values were determined for all of the pork samples. The riboflavin content of pork muscle is low as compared to this tissue's content of thiamine. Pork liver is relatively high in riboflavin content.

LITERATURE CITED

CONNER, R. T., AND G. J. STRAUB 1941 Combined determination of riboflavin and thiamin in food products. J. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 385.

ELLIS, N. R., AND L. L. MADSEN 1942 The thiamin requirements of pigs as related to the fat content of the diet. J. Animal Sci., vol. 1, p. 84.

HENNESSY, D. J. 1942 A standard thiochrome assay for the determination of thiamin in cereal products. The Cereal Chemists' Bull., vol. 2, p. 25.

PYKE, MAGNUS 1940 Vitamin B_1 in vertebrate muscle. Biochem. J., vol. 34, p. 1341.

SCHULTZ, A. S., R. F. LIGHT AND C. N. FREY 1938 Vitamin B_1 metabolism in man. Excretion of B_1 in urine and feces. Proc. Soc. Exp. Biol. and Med., vol. 38, p. 401.

SCHULTZ, A. S., R. F. LIGHT, L. J. CRACAS AND L. ATKIN 1939 The concentration of vitamin B_1 in the tissues of the rat. J. Nutrition, vol. 17, p. 143.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. The Burgess Publishing Co., Minneapolis, Minn., p. 85.

DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

XXXI. FURTHER STUDIES ON THE ROLE OF P-AMINOBENZOIC ACID AND INOSITOL IN LACTATION AND GROWTH OF THE ALBINO RAT[1]

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(Received for publication February 27, 1943)

In 1941 it was shown that as a source of the vitamin B complex, adequate daily doses of pure crystalline thiamine, riboflavin, pyridoxine, choline chloride, calcium pantothemate and the "W" factor from liver extracts resulted in complete failure in lactation of the albino rat. The missing factor, tentatively designated as "Bx", was found in rice polishings, defatted wheat embryo, dried grass, and brewers' yeast, but most abundantly in liver and rice bran extracts (Sure, '41).

Following the suggestion of Ansbacher ('41) that p-aminobenzoic acid may be a component of the vitamin B complex, experiments were carried out to determine if this aromatic acid is a constituent of or identical with the "Bx" factor. Definite responses to p-aminobenzoic acid were obtained in lactation (Sure, '41); these have been now confirmed with numerous additional experiments, the results of which are presented in this communication.

The report of Woolley ('41) that inositol is an essential factor for growth of the mouse, as well as the claim of Pavcek and Baum ('41) that inositol is a growth-promoting substance for the rat, warranted its trial in lactation. However, the pre-

[1] Research paper no. 773 Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Aided by a grant from the Committee on Scientific Research of the American Medical Association.

liminary positive responses in the case of only a few litters of nursing young were not confirmed by much more extensive studies in which inositol was used throughout the growth, reproduction, and lactation periods. The results of this investigation are presented in tables 1 and 2.

TABLE 1

Composition of Sure's salts no. 2.

	gm.		gm.
NaCl	335.0	$MnSO_4$ (anhydrous)	8.0
K_2HPO_4 (anhydrous)	645.0	$ZnCl_2$	0.5
$CaHPO_4.2H_2O$	190.0	$CuSO_4$ (anhydrous)	0.4
$MgSO_4$ (anhydrous)	99.0	$Al_2(SO_4)_3K_2SO_4$	0.4
$CaCO_3$	600.0	NaF	0.5
Ferric citrate (powdered)	55.0	Cobalt chloride	0.5
KI	1.6	Sodium tetra borate (anhydrous) ($Na_2B_4O_7$)	0.5

Before the new series of lactation experiments were initiated, a modification in the salt mixture was made, increasing the $MnSO_4$ from 7.0 to 8.0 gm., and adding cobalt and boron as trace elements, as a factor of safety, to insure optimum mineral requirements, although boron has not yet been shown to be essential for the animal organism. The composition of the new salt mixture, designated as Sure's salts no. 2, is given in table 1. To further insure adequate mineral intake, the new salt mixture was fed at a 5 instead of a 4% level. Because of the high moisture content and the lack of absolute purity, the blood fibrin rations were fed at a 25% plane of intake, which was the equivalent of 20% protein, calculated from the total nitrogen content.

It was realized that the "Bx" factor may be a composite of several unknown components of the vitamin B complex. Therefore, in order to secure a response to a single chemical substance such as p-aminobenzoic acid, small amounts of natural foods or extracts therefrom, were incorporated in some of the rations, i.e., 2% dried grass in ration 13, 2% dried liver in ration 14, and 1% liver concentrate in ration 12. Rations 12, 13, and 14 also contained 0.1% ascorbic acid. Numerous other

TABLE 2

The role of p-aminobenzoic acid and inositol in lactation.

RATION NO.	GROUP NO.	SOURCE OF PROTEIN	SOURCE OF CARBOHYDRATES	SPECIAL ADDITIONS	p-AMINOBENZOIC ACID OR INOSITOL ADDITIONS	NO. OF FEMALES MATED	NO. OF LITTERS	TOTAL YOUNG BORN	YOUNG GIVEN TO REAR	YOUNG REARED	PER CENT YOUNG REARED
13	1	Casein, 10% Fibrin, 10%	Dextrin	Dried grass, 2%	Control	5	6	64	36	6	16.6
13		"	Dextrin	"	inos.[1,2]	5	6	56	35	0	0.0
13		"	Dextrin	"	p.a.b.+inos.[1,2]	5	8	65	41	16	39.0
12	2	Casein, 10% Fibrin, 10%	Dextrin	Liver concentrate, 1%	Control	5	10	127	60	20	33.3
12		"	Dextrin	"	inos.[1]	5	11	96	63	10	15.9
12		"	Dextrin	"	p.a.b.+inos.[1]	5	9	106	54	33	61.0
14	3	Casein, 10% Fibrin, 10%	Dextrin	Dried liver, 2%	Control	5	9	92	40	23	57.5
14		"	Dextrin	"	inos.[1]	5	9	88	53	5	9.4
14		"	Dextrin	"	p.a.b.+inos.[1]	5	11	109	60	51	85.0
8	4	Fibrin 25%	Dextrin	Yeast nucleic acid, 0.2%	Control	5	10	97	59	18	30.5
9		Fibrin, 25%	Dextrin	"	inos., 0.2%	5	6	44	23	5	21.7
10		Fibrin, 25%	Dextrin	"	p.a.b., 0.2%	5	6	67	36	18	50.0
11		Fibrin, 25%	Dextrin	"	p.a.b., 0.2% inos., 0.2%	5	6	50	16	5	31.2
7	5	Casein, 22.5%	Cerelose		Control	10	13	117	77	17	22.1
2	5	Fibrin, 25%	Cerelose		Control	15	32	340	216	87	40.3
3		Fibrin, 25%	Cerelose	Polished rice, 30%		5	11	96	66	44	66.6
4		Fibrin, 25%	Cerelose	"	inos., 0.2%	5	12	112	67	21	31.3
5		Fibrin, 25%	Cerelose	"	p.a.b., 0.2%	5	11	112	63	62	98.4
6		Fibrin, 25%	Cerelose	"	inos., 0.2% p.a.b., 0.2%	5	10	108	60	53	88.3
1		Fibrin, 25%	Dextrin		Control	15	21	195	119	33	27.7

[1] Administered separately from the ration in doses outlined in the text.

[2] p.a.b. = p-aminobenzoic acid; inos. = inositol.

experiments, not included here, showed no special benefits in lactation from ascorbic acid additions. All the rations contained small amounts of nicotinic acid. The cystine additions to rations containing casein were made only during the reproduction and lactation periods. Two sources of carbohydrates were used, a commercial dextrose [2], and dextrin, the latter prepared in the laboratory by cooking cornstarch for 3 to 4 hours at 15 to 17 pounds pressure.

Adequate amounts of the various components of the vitamin B complex were administered separately from the rations in doses outlined in a previous publication (Sure, '41). Vitamins A and D were furnished by 3 drops of halibut liver oil to each animal once weekly until mating, and 3 drops three times weekly during pregnancy and lactation. Vitamin E was provided by wheat germ oil in the rations.

COMPOSITION OF RATIONS

The percentage compositions of diets 1 and 2 were as follows: blood fibrin [3] 25, butterfat 10, carbohydrate 56.95, Sure's salts (no. 2)[4] 5, wheat germ oil 3, and nicotinic acid 0.05. These diets differed merely in that diet 1 had dextrin as the carbohydrate whereas diet 2 had dextrose.[5] Diet 3 was the same as diet 2 except that it contained 30% polished rice with the dextrose reduced accordingly. Diet 4 differed from diet 3 in that the former had 0.2% inositol with a further corresponding reduction in the dextrose. Diet 5 was the same as diet 4 except that no inositol was present but, instead, 0.1% p-aminobenzoic acid; and diet 6 contained both the 0.2% inositol and 0.1% p-aminobenzoic acid, all such additions being at the expense of the dextrose. The p-aminobenzoic acid was increased to 0.2% during the reproduction and lactation periods.

In ration 7 the variable of interest was a commercial vitamin-free casein [6], this constituting 22.5% with a corresponding change in the carbohydrate component of the ration.

[2] Cerelose.
[3] Thoroughly extracted with 95% ethanol.
[4] Table 1.
[5] See footnote 2.
[6] Supplied by the S.M.A. Corporation.

Rations 8, 9, 10 and 11 represented tests of diet 1 with 0.2% yeast nucleic acid and the further addition of either 0.2% inositol or 0.1% p-aminobenzoic acid or both.

Diets 12, 13, and 14 differed from diet 1 in the following respects: instead of 25% fibrin being used as the protein, a combination of 10% fibrin, 10% washed casein [7] and 0.2% cystine was employed. All three diets contained 0.1% ascorbic acid. Diet 12 had 1% liver concentrate, diet 13 had 2% dried grass, and diet 14 contained 2% dried liver, all of these changes from diet 1 being at the expense of the carbohydrate component, which was dextrin.

EXPERIMENTAL

This study was conducted with five groups of animals. Groups 1, 2, and 3 received inositol and p-aminobenzoic acid separately from the ration. Inositol was administered in 10 to 15 mg. daily doses per animal until mating, and in 30 mg. daily doses during the reproduction and lactation periods. p-Aminobenzoic acid was fed in 3 mg. daily doses per animal during the first 4 weeks, followed by 7.5 mg. daily doses until breeding, and 15 mg. daily doses during pregnancy and lactation. Groups 4 and 5 received inositol and p-aminobenzoic acid in the rations, as shown in table 2.

Groups 1, 2, and 3. It will be noted from table 2 that inositol produced a very marked harmful effect on lactation when present in rations containing 2% dried grass, 1% liver concentrate, and 2% dried liver, respectively. On the other hand, p-aminobenzoic acid exerted a most pronounced protective action against inositol injury, as evidenced from efficiency in rearing of young. On ration 13 there was a change from 0 to 39%; on ration 12, from 15.9 to 61.0%; and on ration 14, from 9.4 to 85%.

Group 4. Because of the claim of Nakahara, Inukai, and Ugami ('41) that yeast nucleic acid may be a component of their L_2 factor, the rations in this group of experiments were fortified with 0.2% of such product.[8] In this experimental set-

[7] Thoroughly extracted with acidulated water and dilute ethanol.

[8] Obtained from Eastman-Kodak Co., Rochester, New York.

up p-aminobenzoic acid was added alone (diet 10) and in combination with inositol (diet 11). The injurious effect of inositol is again apparent. The supplementary effect of p-aminobenzoic acid is most marked. However, while there was protection against inositol injury in the presence of the latter, p-aminobenzoic acid, in group 4, did not show its suplementary role.

Group 5. The most surprising results were secured in this group of experiments. Previously, on the basal ration, in the absence of natural foods or the "Bx" factor, the lactation efficiency was about 6% when the protein was furnished by either 20% blood fibrin or by 10% casein and 10% blood fibrin, fortified with 0.2% cystine; also when dextrin was the source of carbohydrates, and when 4% of Sure's salts no. 1 (Sure, '41) provided all the minerals. Ration 2, containing 25% blood fibrin, dextrose as the source of carbohydrates, and 5% of Sure's salts no. 2, allowed 40% success in rearing of young. In other words, this radical change in the basal ration resulted in an increase of efficiency of 34% in lactation. The first experiments with this new basal ration were conducted with five females. This was later extended and results were obtained on fifteen mothers which bore thirty-two litters of 340 young. Another group was then started with fifteen females on the same type of ration but dextrin replaced glucose. While it is not possible at this time to make a fair comparison between the efficiency of glucose versus dextrin for lactation, since the mothers on the glucose diets had second litters while those on dextrin rations had only first litters, still the indications are already apparent that glucose is superior to dextrin for lactation, perhaps by allowing greater efficiency of bacterial synthesis of biotin, folic acid or unknown components of the vitamin B complex (Martin, '42b).

A comparison for lactation was also made between pure vitamin-free casein (diet 7) and blood fibrin (diet 2) using glucose and 5% of Sure's salts no. 2. From the results given in table 2 it is evident that casein, even when fortified with cystine, proved an inferior protein to blood fibrin for lactation.

The substitution of 30% glucose with an equivalent amount of highly milled rice (polished) increased the efficiency of rearing of young from 40.3 to 66.6%. Evidently the polished rice furnished an unknown factor or factors of the vitamin B complex essential for lactation. The addition of 0.2% inositol reduced lactation efficiency by about 50%. However, the addition of 0.2% p-aminobenzoic acid increased lactation efficiency to practically complete success. Also, addition of 0.2% p-aminobenzoic acid to a ration containing 0.2% inositol not only counteracted the harmful effects of the latter but showed almost all of its supplementary effect.

Growth experiments with p-aminobenzoic acid and inositol

The influence of p-aminobenzoic acid on growth of weaned albino rats, with initial weights of 34 to 46 gm., was studied by the paired feeding method on nine pairs of females and twelve pairs of males for 100 to 120 days. This study was carried out on a purified synthetic diet containing 18% vitamin-free casein. The daily dose of the aromatic acid was 3 mg. to the positive control animals. The daily doses per animal of the rest of the components of the vitamin B complex were as follows: 20 μg. thiamine, 20 μg. riboflavin, 20 μg. pyridoxine, 6 mg. choline chloride, 0.5 mg. nicotinic acid, 100 μg. calcium pantothenate, and 10 mg. inositol. The growth was excellent but there was no additional growth produced by the administration of p-aminobenzoic acid. Similarly, inositol addition, in the presence of p-aminobenzoic acid produced no growth responses in seven pairs of females and eleven pairs of males during a period of about 4 months, but there was no injurious effect on growth precipitated by inositol, as has been observed in lactation. The daily dose of inositol to the positive controls was 10 mg. These results are in harmony with those of Unna, Richards and Sampson ('41), Emerson ('41), and Martin ('42a). The reason for p-aminobenzoic acid being essential for lactation and not for growth of the weaned rat is not clear at present, unless there is a difference in the efficiency of synthesis of this substance between very small

nursing young and weaned rats whose capacity for synthesis has been established at that age and weight.

It is of interest to note that Martin ('42a), working with growing animals and the author with lactating animals have noticed dietary disturbances following inositol addition to the vitamin B complex mixture, which however, were counteracted by administration of p-aminobenzoic acid. Martin offers the hypothesis that inositol stimulates the growth of microorganisms which utilized and destroy some member of the vitamin B complex, known or unknown, thus precipitating a deficiency of that factor. It is also possible, however, that excess inositol may interfere with the synthesis of biotin, folic acid, or some other unknown member of the B complex. The response of p-aminobenzoic acid could then be interpreted as supplying a factor essential for synthesis of such substances as biotin and folic acid rather than for neutralization of a toxin (Martin, 42b). The syndrome produced by Martin following addition of p-aminobenzoic acid to the Rockland strain black rat was not confirmed by the author's observations on the weaned or lactating Wistar strain albino rat.

SUMMARY

p-Aminobenzoic acid has a markedly favorable influence on lactation of the albino rat. On the other hand, inositol has a pronounced injurious influence on lactation, which is counteracted by p-aminobenzoic acid.

LITERATURE CITED

ANSBACHER, S. 1941 p-Aminobenzoic acid, a vitamin. Science, vol. 93, pp. 164–165.

EMERSON, G. A. 1941 Failure to cure or prevent greying of rats with p-aminobenzoic acid. Proc. Soc. Exp. Biol. and Med., vol. 47, pp. 448–449.

MARTIN, G. J. 1942 a The interrelationship of p-aminobenzoic acid and inositol. Am. J. Physiol., vol. 136, pp. 124–127.

———— 1942 b "Folic acid" in nutritional achromotrichia. Proc. Soc. Exp. Biol. and Med., vol. 51, pp. 353–355.

NAKAHARA, W., F. INUKAI AND S. UGAMI 1941 Activity of yeast nucleic acid as vitamin L_2. Proc. Imp. Acad. Tokyo, vol. 17, pp. 266–268.

PAVCEK, P. L., AND H. M. BAUM 1941 Inositol and spectacled eye in rats. Science, vol. 93, p. 502.

SURE, B. 1941 Dietary requirements for fertility and lactation. XXIX. The existence of a new dietary factor essential for lactation. J. Nutrition, vol. 22, pp. 499–514.

WOOLLEY, D. W. 1941 Identification of the mouse antialopecia factor. J. Biol. Chem., vol. 139, pp. 29–35.

UNNA, K., G. V. RICHARDS AND W. S. SAMPSON 1941 Studies on nutritional achromotrichia in rats. J. Nutrition, vol. 22, pp. 553–563.

THE PANTOTHENIC ACID REQUIREMENT OF HENS FED A HEATED DIET

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(Received for publication March 8, 1943)

Gillis, Heuser and Norris (’41, ’42) reported that the fertile eggs of hens fed adequate amounts of all known vitamins except pantothenic acid failed to hatch unless the diet was supplemented with this vitamin. Taylor, Thacker and Pennington (’41) presented evidence showing that pantothenic acid has a beneficial effect on embryonic development during the first 14 days of the incubation period. These workers increased the pantothenic acid content of eggs either by direct injection of the vitamin into the egg or by supplementing the normal diet of the hens with additional amounts. Snell and Quarles (’41) studied the vitamin content of eggs during different stages of incubation and concluded that the chick embryo has the ability to synthesize nicotinic acid and inositol but not pantothenic acid and riboflavin.

The results of these studies demonstrate that pantothenic acid is required by the hen for reproduction, but no evidence has been presented showing the amount of the vitamin required for this purpose. Accordingly, studies designed to determine this have been conducted. The results of these experiments are reported in this paper.

EXPERIMENTAL PROCEDURE

The basal diet used in these studies was the same as that reported by Gillis et al. (’42) except that it contained the

equivalent of 1% alcohol soluble liver extract, heated to destroy pantothenic acid. The cereals in this diet were heated at 125°C. for 48 hours. This diet was found to contain 210 μg. of pantothenic acid per 100 gm. by the microbiological assay method of Pennington, Snell and Williams ('40). In this paper, however, the results of the assay will be considered to be 200 μg. per 100 gm. of diet.

White Leghorn pullets in their first year of production were used as the experimental subjects. Studies were made during two experimental periods, the first of 12 weeks' duration and the second of 10 weeks' duration. For the first experiment five lots of pullets were selected for uniformity and placed in wire-floored pens. The basal lot contained thirty pullets and the other lots fifteen pullets each. Males were rotated among the different lots frequently enough to maintain uniformity with respect to breeding. Eggs were incubated weekly and examined for fertility and embryonic mortality on the seventh, fourteenth and eighteenth days of incubation.

The basal diet was supplemented with d-calcium pantothenate in quantities sufficiently great to provide the amount of pantothenic acid per lot as outlined in table 1. The additions to the basal diet were made at intervals of about 5 days in order to keep destruction in storage at a minimum. The hens were changed directly from a normal diet to the experimental diets.

Following the first experimental period of 12 weeks, ten hens from the basal lot were continued on the basal diet. The rest of the hens were again placed on a well-balanced normal diet for 4 weeks. This allowed the hens to recover from any ill-effects due to possible deficiencies resulting from the consumption of the heated diet. At the end of this 4 weeks' period these hens were redistributed into five lots as equally as possible on the basis of the hatchability records of the previous experiment. The management of the hens and the experimental procedure used in this experiment were the same as in the previous one.

TABLE 1

Percentage hatchability of fertile eggs from hens fed different levels of pantothenic acid.

WEEK AND HATCH	MICROGRAMS OF PANTOTHENIC ACID ADDED PER 100 GM. OF BASAL DIET						
	None	250	500	1000	1500	2000	2500
	%	%	%	%	%	%	%
			Experiment 1				
1	79(44) [1]	69(16)	80(5)	74(31)	——	70(29)	——
2	68(75)	80(34)	76(37)	62(34)	——	70(41)	——
3	56(57)	38(29)	47(46)	69(31)	——	72(39)	——
4	37(73)	33(33)	58(45)	60(15)	——	70(32)	——
5	33(72)	37(32)	64(56)	60(34)	——	83(40)	——
6	20(44)	21(38)	67(55)	58(33)	——	85(35)	——
7	10(63)	32(40)	53(55)	71(35)	——	71(42)	——
8	10(60)	37(30)	55(51)	63(38)	——	69(35)	——
9	7(46)	25(24)	66(47)	65(34)	——	65(30)	——
10	8(24)	20(25)	61(31)	76(33)	——	74(27)	——
11	9(12)	12(16)	48(25)	69(36)	——	79(19)	——
12	11(60)	26(31)	55(47)	57(37)	——	67(27)	——
Ave. (after depletion [2])	9.2	28.1	57.4	64.8	——	73.5	——
WEEK AND HATCH	NONE	250	500	1000	1500	2000	2500
			Experiment 2				
1	4(28)	——	60(30)	95(23)	67(27)	82(34)	77(31)
2	4(26)	——	55(29)	82(23)	65(26)	70(27)	89(27)
3	17(23)	——	67(31)	71(28)	80(30)	71(24)	80(35)
4	20(10)	——	54(24)	70(20)	81(31)	65(20)	77(28)
5	9(22)	——	67(27)	75(20)	79(24)	80(20)	77(30)
6	8(13)	——	56(25)	54(17)	90(21)	75(24)	69(29)
7	8(25)	——	33(27)	50(18)	74(23)	69(26)	71(32)
8	3(33)	——	16(37)	40(20)	48(27)	62(21)	49(35)
9	15(27)	——	22(37)	22(22)	58(26)	52(27)	47(19)
10	8(26)	——	27(37)	32(22)	45(22)	42(12)	43(21)
Ave. (3-10 inclusive)	11.0	——	42.8	51.8	69.4	64.5	64.2

[1] Data in parentheses represent number of fertile eggs incubated.

[2] Hatches 7–12, inclusive, for the lot not receiving pantothenic acid; for other lots hatches 3–12, inclusive.

RESULTS AND DISCUSSION

The results of feeding different levels of pantothenic acid on the hatchability of fertile eggs are summarized in table 1. In the first experiment the hatchability of the fertile eggs from the basal lot declined from 79% to about 10% in 6 weeks. After this there was no further significant decrease. This shows that the hens were completely depleted of their body reserves of pantothenic acid within this time. The average hatchability of the last 6 weeks, therefore, is taken as the average for this lot. All other lots received supplementary pantothenic acid and, hence, the amount stored in the eggs became stationary earlier. An inspection of the data indicates that the hens were significantly influenced by their previous dietary regime for only about 2 weeks. It was decided, therefore, to use the last 10 weeks of this experiment as the basis of the average figures for the other lots.

The decline in hatchability of the fertile eggs of the lot receiving the addition of 250 μg. of pantothenic acid was not quite as marked as in the basal lot, but it was so much below that of the other lots that there was no doubt that the 250 μg. of supplementary pantothenic acid was inadequate to maintain the hatching power of the eggs. The average hatchability of eggs from the three lots receiving the higher pantothenic acid additions was correlated with the level of pantothenic acid in the diet, the best hatchability being secured from the lot receiving the most pantothenic acid.

Although the differences in average hatchability of the three lots receiving the higher pantothenic acid additions appeared to be significant, it was felt advisable to examine the results statistically. This treatment showed a very significant difference between these lots. The data were also tested statistically for regression and were found to exhibit a significant regression. These results tentatively placed the optimum pantothenic acid requirement for reproduction in the hen above 1200 μg. per 100 gm. of the diet. This is in agreement with a previous experiment in which the authors ('42), with-

out attempting to determine quantitative requirement, secured better hatchability when the hens received a supplement of 2000 μg. of calcium pantothenate per 100 gm. of diet, than when the supplement was 1000 μg. per 100 gm.

The second experiment was designed to establish the quantitative requirements within a narrower range, and especially to establish an upper limit which the preceding experiment failed to do.

The results of experiment 2, as given in table 1, also showed that the pantothenic acid requirement for reproduction is above 1200 μg. per 100 gm. of the diet, thus being in accord with experiment 1. The average hatchability of the eggs from the lot receiving 1200 μg. of pantothenic acid per 100 gm. of diet was below the three lots receiving more pantothenic acid, although at the beginning of the period it had the highest hatchability of any lot. It is also clear from the results of this experiment that the optimum pantothenic acid requirement for hatchability does not lie above 1700 μg. per 100 gm. of diet. The hatchability of eggs from the lot receiving 1700 μg. was slightly better than that of the lots receiving more pantothenic acid.

The quantitative pantothenic acid requirement for maximum hatchability of fertile eggs in hens fed the diet used in these studies, therefore, is between 1200 and 1700 μg. per 100 gm. of diet. Average daily food consumption was 110 gm. per hen, which makes the average daily requirement per hen between 1320 and 1870 μg. The average weight of the hens was 1707 gm. On the basis of body weight the requirement would thus be between 775 and 1000 μg. per kilogram of hen per day. The fact should not be overlooked, however, that the requirement may be less if hens are fed a more nearly normal diet of unheated feedstuffs, since a sufficiency of other necessary factors may exercise a sparing effect on pantothenic acid.

The basal diet used in these studies appeared to be deficient in some factor (or factors) required for reproduction other than pantothenic acid. This deficiency became serious, however, only after the hens had been maintained on this diet

for a prolonged period of time. The rapid drop in hatchability of all lots during the last few weeks of the second experiment is evidence of the existence of this latent deficiency. The decline was too great to be accounted for on the basis of seasonal variations alone. Cravens, Sebesta, Halpin, and Hart ('42) have recently reported biotin to be essential for hatchability. Since biotin has been shown by Ringrose and Norris ('36) and by others to be slowly destroyed by dry heat treatment, it is possible that the diet may also have been partially deficient in this factor.

The effect of pantothenic acid on the viability of the chicks hatched in both experiments was marked. The chicks hatched from lots receiving less than 1200 μg. of pantothenic acid were inferior. They usually exhibited general debility, muscular incoordination, swollen hocks, and poor down quality. Many of the embryos had great difficulty in breaking through the shell and died soon afterwards. When chicks from deficient lots were placed on an adequate diet mortality was extremely high. On the other hand, the chicks hatched from the lots receiving more adequate levels of pantothenic acid had little mortality and grew vigorously when given an adequate diet.

The peak of embryonic mortality in the lot fed the basal diet occurred near the end of the incubation period. In this lot 79% of all embryonic deaths occurred after the fourteenth day, and 52% occurred after the eighteenth day. Up to the fourteenth day the differences in the total percentages of dead embryos in the different lots were slight. The mortality of the embryos from lots receiving adequate pantothenic acid was more uniformly distributed over the incubation period.

The effects of the different levels of pantothenic acid on egg production were also studied. During the first experiment the average rate of egg production was best when the hens received a supplement of 500 μg. or above. During the second experiment the pantothenic acid content of the diet could, in no way, be correlated with rate of egg production. It is impossible to say from these results, therefore, whether or not pantothenic acid influences egg production, but it appears

that, in any case, 700 μg. per 100 gm. of diet is sufficient for this function.

The mortality and body weight of the hens were not influenced by the level of pantothenic acid in the diet. Apparently the requirement for maintenance in the mature hen is not greater than the pantothenic acid content of the basal diet which was approximately 200 μg. per 100 gm. This observation indicates that the pantothenic acid requirement of chickens decreases with advancing age, as Bauernfeind, Norris and Heuser ('42) found that the quantity of pantothenic acid required for chick growth is about 600 μg. per 100 gm. of diet. Waisman, Mills and Elvehjem ('42) reported that the pantothenic acid requirement for maximum chick growth is 750 μg. per 100 gm. of diet. These results are in agreement with the conclusion of Unna and Richards ('42), that the amount of pantothenic acid required by the adult rat is not more than 25% of that needed for early growth.

CONCLUSIONS

Hens fed a diet consisting principally of heated feedstuffs supplemented with known necessary minerals and vitamins except pantothenic acid were found to require between 1200 and 1700 μg. of this vitamin per 100 gm. of diet in order to maintain optimum reproduction. The pantothenic acid requirement for egg production was found to be not more than 700 μg. per 100 gm. of diet and may be considerably less. Maintenance of weight and livability were satisfactory on the basal diet which contained 200 μg. of pantothenic acid per 100 gm.

The basal diet used in these studies was found to be deficient in a factor (or factors) required for reproduction other than pantothenic acid. The deficiency affected reproduction detrimentally, however, only after the hens had been maintained on the diet for a prolonged period of time. Whether or not the deficiency influenced the requirement for pantothenic acid could not be determined from the evidence.

LITERATURE CITED

BAUERNFEIND, J. C., L. C. NORRIS AND G. F. HEUSER 1942 The panothenic acid requirement of chicks. Poultry Sci., vol. 21, pp. 142–146.

CRAVENS, W. W., E. E. SEBESTA, J. G. HALPIN AND E. B. HART 1942 Effect of biotin on reproduction in the domestic fowl. Proc. Soc. Exp. Biol. and Med, vol. 50, pp. 101–104.

GILLIS, M. B., G. F. HEUSER AND L. C. NORRIS 1941 The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl. (Abstract) Poultry Sci., vol. 20, p. 460.

———— 1942 The need for pantothenic acid and an unidentified factor in reproduction in the domestic fowl. J. Nutrition, vol. 23, pp. 153–163.

PENNINGTON, D., E. E. SNELL AND R. J. WILLIAMS 1940 An assay method for pantothenic acid. J. Biol. Chem., vol. 135, pp. 213–222.

RINGROSE, A. T., AND L. C. NORRIS 1936 Differentiation between vitamin G and an insoluble factor preventing a pellagra-like syndrome in chicks. I. J. Nutrition, vol. 12, pp. 535–552.

SNELL, E. E., AND E. QUARLES 1941 The effect of incubation on the vitamin content of eggs. J. Nutrition, vol. 22, pp. 483–489.

TAYLOR, A., JUANITA THACKER, AND DOROTHY PENNINGTON 1941 The effect of increased pantothenic acid in the egg on the development of the chick embryo. Science, vol. 94, pp. 542–543.

UNNA, KLAUS, AND G. N. RICHARDS 1942 Relationship between pantothenic acid requirement and age in the rat. J. Nutrition, vol. 23, pp. 545–553.

WAISMAN, H. A., R. C. MILLS AND C. A. ELVEHJEM 1942 Factors required by chicks maintained on a heated diet. J. Nutrition, vol. 24, pp. 187–197.

THE INADEQUACY OF EIGHT SYNTHETIC B VITAMINS FOR THE NUTRITION OF PUPPIES — UNKNOWN FACTOR (FACTORS) IN YEAST AND PROBABLY LIVER [1] [2]

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ONE FIGURE

(Received for publication March 13, 1943)

This investigation was made in an attempt to determine whether dogs would grow normally on a diet containing only the chemically pure vitamins available at the time the work was done (1941–42). In some previous unpublished work it was noted that adult dogs developed ulcers in the mouth and on the leg joints while subsisting on similar diets. Since these dogs bore jejunostomies, there was the possibility that the lesions were the result of discharges from the stoma of acid chyme which was incompletely removed by licking. Leg joint lesions were demonstrated by Cowgill, Stucky and Rose ('29) in dogs deprived of the entire vitamin B-complex. In order to get further information concerning the inadequacies of this diet, it was fed to puppies from the time of weaning.

METHODS

Seven mongrel puppies from three litters were used in this investigation; dogs 1 and 2 were from one litter, dogs 3 to 6, inclusive, from a second, and dog 7 from a third litter. The

[1] Taken from a thesis presented by J. P. Lambooy to the Division of Graduate Studies, University of Rochester, in partial fulfillment of the requirements for the degree Doctor of Philosophy, 1942.

[2] Supported in part by a grant from Standard Brands, Inc.

puppies received the diets shown in table 1. The salt mixture was that of Arnold and Elvehjem ('38) except that cobalt was added in such quantities that each dog received 0.1 mg. per day (Frost, Elvehjem and Hart, '41).

The vitamin contents in milligram per gram of the dried foil yeast [3] were as follows: thiamine 0.108; riboflavin 0.040; nicotinic acid 0.259; pyridoxine 0.020; and pantothenic acid 0.180.

The casein was freed of vitamin B-complex by repeated extractions with 0.1% acetic acid and ethyl alcohol. The extraction was assumed to be complete when the fluorescence due to riboflavin disappeared from the filtrates viewed in ultra-violet light in the absence of visible light. The sucrose was extracted twice with 80% (by weight) ethyl alcohol and washed after each extraction with a like mixture while on the filter.

A diet containing the casein and sucrose, extracted according to these methods, was compared in growth studies on rats with an identical diet made with a commercial vitamin B-complex-free casein.[4] The paired feeding technic revealed the diets to be indistinguishable and free of vitamin B-complex.

The protein of yeast in the basal diet was replaced by casein in the experimental diet. During the experimental period the animals received a water solution or suspension of the synthetic crystalline B-vitamins in amounts roughly equivalent to the 10% high vitamin yeast supplement in the basal diet. Some of the vitamins were fed in somewhat larger or smaller amounts than furnished by the yeast to produce better agreement between the supplement and the published requirements for the dog.

All the puppies used in this investigation were weaned at the age of 6 weeks and placed on their respective diets. During the early part of the experiment the dogs were immunized against distemper, dewormed and freed of fleas.

[3] Fleischmann's.

[4] S. M. A. Corporation, Chagrin Falls, Ohio.

RESULTS

Dog 1 was maintained on the basal diet for 151 days and at no time showed any signs of deficiency. He was exceptionally active, always ate willingly, and grew at a regular and uniform rate. On the 151st day he was placed on the experimental diet and maintained thereon for 105 days (fig. 1). Near the 75th

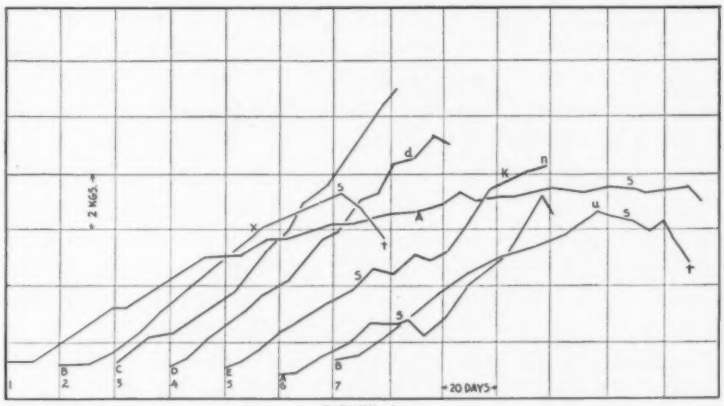

FIGURE 1
WEIGHT CURVES OF PUPPIES 1-7 INCLUSIVE.(KILOGRAMS)

Fig. 1 Growth curves of dogs, fed synthetic diets containing all fat-soluble vitamins and synthetic B factors, with and without yeast or liver filtrate concentrates.

Dog 1 A, placed on experimental diet A, containing all eight synthetic B factors; s, scaliness of skin appeared.

Dog 2 B, placed on experimental diet B, i.e., containing all synthetic B factors shown in table 1, except inositol and p-aminobenzoic acid, plus yeast fraction II of Osborne and Wakeman ('19); x, yeast fraction discontinued; s, scaliness of skin and diarrhea appeared; + death.

Dog 3 C, dog placed on experimental diet C, i.e., all eight synthetic factors, plus yeast filtrate factor of Koehn and Elvehjem ('37) — no deficiencies.

Dog 4 D, dog placed on experimental diet D, i.e., all eight synthetic factors and a filtrate factor from liver (Koehn and Elvehjem '37); d, skin dry and dusty but not scaly.

Dog 5 E, placed on experimental diet E, i.e., double amounts of all eight synthetic B factors shown in table 1; s, scaliness of skin appeared and intermittent diarrhea; k, placed on kitchen scraps; n, skin normal.

Dog 6 A, dog on diet A throughout; s, scaliness of skin and diarrhea continuous and severe, + death.

Dog 7 B, placed on diet B without filtrate factor; u, ulcerous dermatitis particularly on ventral surfaces. At this point inositol and p-aminobenzoic acid added; dermatitis persisted; s, scaliness of skin became general after 102nd day; diarrhea throughout entire time; + death.

day on the experimental diet the dog became coprophagous, showed loss of hair and a small amount of scaliness of the skin. At the end of the basal period the hematocrit was 34% and the hemoglobin concentration 14.9 gm. per 100 ml. of blood. During the last 75 days the dog received 200 mg. of iron in the form of ferric ammonium citrate in addition to that furnished by the salt mixture in the diet. On the 100th day after starting the experimental diet the hematocrit was 42% and the hemoglobin concentration 17.0 gm. per 100 ml. of blood.

Dog 2 received the experimental diet minus inositol and p-aminobenzoic acid. An additional supplement was given in the form of a yeast fraction prepared by the method outlined for fraction no. II, by Osborne and Wakeman ('19). This fraction was fed in amounts equivalent to twice the yeast received by dog 1. On the 73rd day the Osborne-Wakeman fraction was discontinued and thereafter the dog showed a decline in rate of growth (x, fig. 1). On the 102nd day the dog's eyes began to discharge a great deal of mucoid material, but abnormal secretions from mouth and nose were absent. At this time the dog also began to suffer from intermittent diarrhea. The skin had become so dry and scaly that the coat of hair was very dusty (s, fig. 1). From the 102nd day until the 118th day the dog continued to eat about half of its usual dietary intake and lost weight rapidly. During this period of rapid weight loss inositol and p-aminobenzioc acid were added to the vitamin supplement. On the 117th day the hematocrit was 27% and the hemoglobin concentration 10.8 gm. per 100 ml. of blood.

Dog 3 received the experimental diet plus an additional supplement of a yeast concentrate prepared according to the method for the preparation of the filtrate factor as outlined by Koehn and Elvehjem ('37). The starting material was 400 gm. of 50% alcohol (by weight) — soluble extract of dry yeast. The daily dose was equivalent to 20 gm. of the original yeast. Dog 3 showed the best rate of growth of all the dogs. At no time did any of the previously mentioned signs of deficiency appear. The skin was clean and the hair clean and glistening. The yeast concentrate, therefore, contains the additional fac-

tor or factors required for normal growth and maintenance of healthy skin. This dog was also fed daily 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. On the 89th day the hematocrit was 31% and the hemoglobin concentration 12.0 gm. per 100 ml. of blood.

Dog 4 received the experimental diet plus an additional supplement of a liver concentrate which was made from 400 gm. of 95% alcohol-soluble liver extract as outlined by Koehn and Elvehjem for the preparation of the filtrate factor. The daily dose was equivalent to 4 gm. of the original liver extract. This dog also received daily 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. This dog showed a good rate of growth until about the 82nd day, at which time the rate began to decrease a little. About the 90th day it was observed that although the skin could not be considered scaly, it was dry and dusty (d, fig. 1). The coat of hair was still in excellent condition. Apparently a liver fraction prepared in this manner does not contain sufficient amounts of the missing factor or factors. On the 89th day this dog had a hematocrit of 33% and a hemoglobin concentration of 12.2 gm. per 100 ml. of blood.

Dog 5 received the experimental diet with double the dosage of vitamins shown in table 1. This dog also received an additional daily supplement of 200 mg. of iron as ferric ammonium citrate for the last 75 days of the experiment. About the 50th day of the experiment dry, scaly skin and dusty hair were observed. There were frequent interludes of remittant diarrhea. The areas which had been clipped for the distemper immunization injections showed a very slow rate of hair growth and there was definite achromotrichia. This was also found to be true of dogs 6 and 7, but not the other dogs. After 103 days on the diet this dog was changed to a diet of kitchen scraps (n, fig. 1) and in the short period of 2 weeks the skin was clean and the hair glistening; the clipped areas showed considerable new pigmented hair growth. On the 89th day the hematocrit was 31% and the hemoglobin concentration 12.0 gm. per 100 ml. of blood.

Dog 6 received the experimental diet and at no time appeared to be healthy. This dog also received daily 200 mg. of iron as ferric ammonium citrate. The dermatitis of dog 6 was not as severe as that of dog 7, but there was dryness and scaliness of skin, and the hair was dull, exceptionally dusty and sparse. This dog suffered from almost constant diarrhea. His mature appearance and behavior were marked characteristics.

TABLE 1

Composition of diets and vitamin supplements.

	BASAL	EXPERIMENTAL
Casein	25%	35%
Sucrose	41%	41%
Hydrogenated cottonseed oil	18%	18%
Bone ash	4%	4%
Salts	2%	2%
Yeast (dry)[1]	10%	0%
Thiamine chloride hydrochloride[2]	0	0.050 mg./kg./day
Riboflavin	0	0.100 mg./kg./day
Nicotinic acid	0	0.500 mg./kg./day
Ca-d-pantothenate	0	0.220 mg./kg./day
Pyridoxine	0	0.040 mg./kg./day
Choline chloride	0	0.200 mg./kg./day
Inositol	0	0.500 mg./kg./day
p-Aminobenzoic acid	0	0.500 mg./kg./day
Vitamin A (concentration; 200,000 I.U./gm.)	200 I.U./kg./day	200 I.U./kg./day
Vitamin D (viosterol in oil)	10 I.U./kg./day	10 I.U./kg./day
Vitamin E	1.0 mg./kg./day	1.0 mg./kg./day
Vitamin K	1.0 mg./kg./day	1.0 mg./kg./day

[1] Kindly furnished by Standard Brands, Inc.

[2] All vitamins except A, D, inositol and p-aminobenzoic acid were kindly furnished by Merck and Company, Rahway, New Jersey.

On the 96th day this dog showed little interest in his food but retained his normal, although mild, degree of activity. On the 98th day he died and autopsy revealed the thymus gland heavily spotted with hemorrhagic areas about 2 to 5 mm. in diameter. A similar observation was made by McKibbin et al. ('39), Schaefer et al. ('42) and Morgan and Simms ('40a,

'40b), and ascribed to pantothenic acid deficiency because prophylaxis was achieved by the administration of 0.1 mg. of this vitamin per kilogram per day. Dog 6 received 0.22 mg. of Ca-d-pantothenate per kilogram per day which is apparently more than the growing dog requires. On the 89th day the hematocrit was 31% and the hemoglobin concentration 11.7 gm. per 100 ml. of blood.

Dog 7 received the experimental diet, except that inositol and p-aminobenzoic acid were omitted. About the 10th day, dog 7 showed small ulcers on the ventral surface of its body; these disappeared on the 82nd day. On the 96th day new areas appeared and dermatitis was general (u, fig. 1). At this time the dog began to lose weight and the vitamin supplement was changed by the inclusion of inositol and p-aminobenzoic acid. On the 102nd day the dermatitis was extensive and the dog's eyes began to discharge considerable amounts of mucoid material. On the 120th day the whole ventral surface and the inside areas of the dog's legs were spotted with small ulcers. The rest of the animal's skin was in a dry, scaly and dusty condition. The dog's coat of hair, which until the 102nd day was glistening, had become dull and dirty by the 120th day. During the whole experiment the dog suffered from diarrhea. Dog 7 died on the 127th day; autopsy failed to reveal any specific cause for death. On the 120th day the hematocrit was 23% and hemoglobin concentration 7.1 gm. per 100 ml. of blood.

DISCUSSION

The above investigation strongly suggests that the lesions observed in the adult jejunostomized dogs were due to leakage from the stoma. Neither the leg joint lesions nor the mouth lesions were found in any of the puppies, but there is the possibility that this difference in response might have been due to the difference in the ages of the experimental animals. That the lesions might have been due to a deficiency of inositol or p-aminobenzoic acid seems to be ruled out on the basis of their failure to appear in dogs 2 and 7. The small lesions seen in

dog 7 were not the same as seen in the adult dogs and were not confined to the joints.

It appears that there is in yeast one or more substances concerned with the maintenance of healthy skin and hair in dogs. Morgan ('41) reported that a young dog fed a synthetic diet supplemented with a vitamin B-complex containing thiamine chloride hydrochloride, riboflavin, pyridoxine, pantothenic acid, and nicotinic acid, grew well and showed no greying hair after 6 months on the diet. However, she reports that this dog had dull powdery hair. Morgan used wheat germ oil instead of synthetic α-tocopherol as a source of vitamin E, and choline chloride, inositol, and p-aminobenzoic acid as such were included.

Schaefer, McKibbin, and Elvehjem ('42) demonstrated that when a diet containing acid and alcohol-extracted casein, supplemented with thiamine chloride hydrochloride, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and choline chloride, was fed to young dogs a deficiency characterized by anorexia and loss of weight appeared after 1 to 3 months. They also observed greying of hair in some dogs. They state that addition of inositol, p-aminobenzoic acid or glutamine had little or no effect on these symptoms. These workers do not report the occurrence of dermatitis in their animals.

Since the animals used in this investigation received far less choline than administered by Schaefer, McKibbin and Elvehjem ('41) it may be argued that they lacked this factor. Some of our dogs showed weight losses at about the same time after the experimental diets were begun as did those reported by these other observers. The additional fact must not be overlooked that the animals used in this investigation received 35% of their diet as casein. On the basis of the onset of weight loss these two experiments seem comparable. Furthermore, gross examination of the dogs that came to autopsy showed no abnormalities in the livers.

Those dogs showing the most severe dermatitis, dusty hair and diarrhea were the ones which lost weight and died. Since pronounced diarrhea was observed in only those dogs receiv-

ing the quantities of vitamins listed in table 1, and was not an outstanding characteristic of the dog receiving twice these amounts, the suggestion is made that although the quantities administered probably exceeded the minimal requirements, they may not be optimal for all of the components. The vitamin supplement was lower than the amounts in the yeast of the basal diet by three-fourths in thiamine chloride hydrochloride, and by one-half in pantothenic acid. The feeding of 0.5 mg. per kilogram per day of inositol and p-aminobenzoic acid was purely arbitrary since little was known concerning the dog's requirements for these compounds.

The above observations seem to justify the conclusion that the factor or factors responsible for the maintenance of good health of the skin and hair in growing dogs is found in yeast and probably liver, and that the development of signs of deficiency disease is not due to a deficiency of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, inositol or p-aminobenzoic acid. Furthermore, it appears to be essential to make thorough extractions of both protein and carbohydrate components of the diet in experiments of this type.

SUMMARY

Puppies were fed synthetic diets supplemented with a synthetic vitamin B-complex with or without additional vitamin-rich concentrates.

The young dogs receiving the synthetic vitamin B-complex containing only thiamine chloride hydrochloride, riboflavin, nicotinic acid, Ca-d-pantothenate, pyridoxine, choline, inositol and p-aminobenzoic acid developed varying degrees of dermatitis, loss of hair and general unhealthy skin and coat in 75 to 125 days, and died in 100 to 150 days.

If dry whole yeast or concentrates of yeast or liver were fed in addition to the crystalline vitamins the deficiency failed to appear.

The authors take this opportunity to express their appreciation to Miss Betty Robinson for technical assistance.

LITERATURE CITED

ARNOLD, A., AND C. A. ELVEHJEM 1938 Influence of the composition of the diet on the thiamine requirement of dogs. Am. J. Physiol., vol. 126, p. 289.

COWGILL, G. R., C. J. STUCKY AND W. B. ROSE 1929 The physiology of vitamins. V. Cutaneous manifestations related to a deficiency of the vitamin B complex. Arch. Pathol., vol. 7, p. 197.

FROST, D. V., C. A. ELVEHJEM AND E. B. HART 1941 A study for the need of cobalt in dogs on milk diets. J. Nutrition, vol. 21, p. 93.

KOEHN, C. J. JR., AND C. A. ELVEHJEM 1937 Further studies on the concentration of the antipellagra factor. J. Biol. Chem., vol. 118, p. 693.

MCKIBBIN, J. M., R. J. MADDEN, S. BLACK AND C. A. ELVEHJEM 1939 The importance of vitamin B_6 and factor W in the nutrition of dogs. Am. J. Physiol., vol. 128, p. 102.

MORGAN, A. F., AND H. D. SIMMS 1940a Greying of fur and other disturbances in several species due to a vitamin deficiency. J. Nutrition, vol. 19, p. 233.

———— 1940b Anti-grey hair vitamin deficiency in the silver fox. J. Nutrition, vol. 20, p. 627.

MORGAN, A. F. 1941 The effect of imbalance in the "filtrate fraction" of the vitamin B complex in dogs. Science, vol. 93, p. 261.

OSBORNE, T. B., AND A. J. WAKEMAN 1919 Extraction and concentration of the water-soluble vitamine from brewers' yeast. J. Biol. Chem., vol. 40, p. 383.

SCHAEFER, A. E., J. M. MCKIBBIN, AND C. A. ELVEHJEM 1941 Studies on the vitamin B complex in the nutrition of the dog. J. Nutrition, vol. 23, p. 491.

———— 1942 Pantothenic acid deficiency studies in dogs. J. Biol. Chem., vol. 143, p. 321.

ANTI-ACRODYNIC POTENCY OF SEED OILS[1,2]

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(Received for publication March 19, 1943)

The curative effect of certain oils on rat acrodynia has been demonstrated by Birch and György ('36), Richardson and Hogan ('36), Quackenbush, Platz and Steenbock ('39), and others. Linoleic acid was an active ingredient in these oils while linolenic acid was apparently inactive since linseed oil, containing a total of 70% linoleic and linolenic acids, was much less curative than corn, cottonseed, or wheat germ oil with a total of 40 to 55% linoleic acid and no linolenic acid. However, it was possible that fatty acids other than linoleic might be curative and that certain oils might be more potent than any yet studied.

The work presented in this paper was an attempt to correlate the observed anti-acrodynic potency of various seed oils with the linoleic acid content as determined on identical samples. It does not deal with the cure of the less severe dermatitis of Burr and Burr ('29).

EXPERIMENTAL

I. Methods and techniques

The technique employed for determining the potency of the oils was that previously developed in this laboratory (Quackenbush, Platz and Steenbock, '39). Female rats and their

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station, Madison.

[2] We express our appreciation to The Lever Brothers Company for a grant in support of this work.

litters were placed on a diet consisting largely of potatoes until the young attained a weight of 40 gm. The young were then transferred to a diet having the following percentage composition: glucose [3] 78, water and alcohol-extracted casein [4] 18, and salts 4. This was supplemented daily with the following vitamins: thiamine 10 μg., riboflavin 20 μg., carotene 10 μg., and calciferol 1 μg. per rat per day. With this diet (diet V) acrodynia was produced in our specially prepared rats in 5 to 6 weeks.

The severity of the dermatitis at each of the loci of incidence (mouth, eyes, paws, ears and tail) was evaluated on a scale of four. The total score on each animal was then used to give the dermal index (Quackenbush, Steenbock, Kummerow and Platz, '42).

When an animal had a dermal index of 5 to 7 it was given a daily supplement of the oil to be tested. After 3 weeks an animal was considered cured if the lips, eyes and ears were normal in appearance and the paws and tail were free from all symptoms except a dry scaliness. This was equivalent to a dermal index of 2 or less. When a dose produced a dermal index of 2 or less in more than 50% of the rats in the group it was accepted as being curative.

All the oils were fed daily in amounts equivalent to 12 mg. of linoleic acid. This was considered ample since the minimum curative level of linoleic acid in the form of the ethyl ester had been found to be between 5 mg. and 10 mg. per rat per day (Quackenbush, Platz and Steenbock, '39). Some of the oils which were curative at the 12 mg. level were later fed in amounts equivalent to 4 mg. of linoleic acid.

The linoleic acid content of most of the oils was determined by a method developed in this laboratory (Anthony, Quackenbush and Steenbock, '43). For the remaining analyses we are indebted to Schuette and co-workers ('30, '32, '34a, '34b, '37, '40a, '40b, '40c, '41). Analysis of identical samples was considered necessary since the assays of some of the oils

[3] Cerelose.

[4] Labor for purification of the casein was furnished by the Works Progress Administration.

TABLE 1

The effect on rat acrodynia of seed oils of known composition.[1]

OIL	LINOLEIC ACID	LINOLENIC ACID	AMOUNT OF OIL FED					
			Equivalent to 12 mg. linoleic acid			Equivalent to 4 mg. linoleic acid		
			No. rats	No. cured	Average dermal index	No. rats	No. cured	Average dermal index
	%	%						
Tobacco [2]	81	0	3	3	1			
Hackberry [2]	70	0	4	4	1			
Muskmelon	70	0	4	3	2	4	1	3
Watermelon	66	0	7	6	2	3	1	2
Poppy	65	0	6	4	2	7	2	4
Raisin	63	0	5	5	1	4	3	2
Cucumber	61	0	4	3	2	3	0	4
Elderberry [2]	58	24	6	3	3			
Sunflower	53	1	5	3	2			
Pumpkin	49	0	7	5	2	1 [3]	1	2
Squash	49	0	8	7	2	2	0	4
Rye germ [2]	42	5	3	3	2			
Sesame	39	3	3	3	1	2	0	8
Fenugreek [2]	37	19	5	2	3			
Clover	36	18	6	4	2	3	1	4
Highbush cranberry [2]	36	0	4	3	2			
Hemp [4]	31	33	5	2	2	3	0	3
Oat germ [5]	31		4	4	1			
Linseed [4]	27	39	4	2	3			
Coffee bean [2]	25	0	4	4	1			
Alfalfa	22	42	6	3	3	11	4	4
Brazilnut [2]	21	0	4	4	1			
Perilla [4]	18	65	4	0	4			
Elm [2]	8	0	4	3	2			

[1] In the computation of this table negative results obtained on animals which contracted "respiratory" infections or which weighed less than 45 gm. when given the supplements were not included because such animals frequently failed to respond to supplements of known potency.

[2] We are indebted to Dr. H. A. Schuette and co-workers for these samples and their analyses.

[3] Four of the rats put on experiment died early. They all had a severe acrodynia (average dermal index 5).

[4] These were also assayed at 36 mg. equivalency. Hempseed oil was curative (average dermal index of 2) while linseed and perilla oil were non-curative (average dermal index of 4 and 3, respectively).

[5] Oat germ oil not analyzed. Analyses listed are from the literature (Amberger and Hill, '27).

reported in the literature were found to vary over a wide range (Hilditch, '40; Dean, '38).

II. Results

Oils fed in an amount equivalent to 12 mg. of linoleic acid consistently cured acrodynia unless the oil contained a considerable amount of linolenic acid (table 1). The presence of linolenic acid interfered with the curative action as shown by the data on linseed, alfalfa, perilla, elderberry and fenugreek seed oils. When these oils were fed in amounts greater than the 12 mg. equivalency, the average dermal index was not reduced correspondingly. Hempseed and perilla oils fed at a level equivalent to 36 mg. of linoleic acid were only slightly more curative than at the 12 mg. level, while linseed oil actually was less curative at 36 mg. than at 12 mg. A few tests on marine oils indicated that lipids other than linolenic acid also interfered with the cure of acrodynia. In fact, shark liver, herring, seal, and whale oils aggravated the symptoms to such an extent that the average dermal indices of the groups at 3 weeks ranged from 8 to 15.

With the exception of the instances cited above, the oils had a potency approximately equivalent to their linoleic acid content. Doses equivalent to 12 mg. were curative while doses equivalent to 4 mg. were not curative in eleven out of twelve oils tested. The one exception was raisin seed oil, but the data with this oil are too limited to warrant final conclusions.

SUMMARY

1. Twenty-four seed oils of known linoleic and linolenic acid content were assayed for their potency in the cure of rat acrodynia.

2. The anti-acrodynic potency of the oils was found to be in direct proportion to their linoleic acid content unless they contained considerable amounts of linolenic acid.

3. When linolenic acid was present the curative effect of the oils was greatly reduced. Isolated data on marine oils

suggest that other lipids besides linolenic acid also produce this effect.

4. When linolenic acid was absent, or when it was present only in minor proportions, a daily dose equivalent to 12 mg. of linoleic acid cured the acrodynia; 4 mg. was not curative.

LITERATURE CITED

AMBERGER, K., AND E. W. HILL 1927 Uber die Zusammensetzung des Haferöls. Z. Untersuch. Lebensm., vol. 54, p. 417.

ANTHONY, D. S., F. W. QUACKENBUSH AND H. STEENBOCK 1943 Crystallization at low temperatures as a technique in the analysis of oils. Oil and Soap, vol. 20, p. 53.

BAIN, JAMES A., AND H. A. SCHUETTE 1940a Unpublished data.

BIRCH, T. W., AND P. GYÖRGY 1936 A study of the chemical nature of vitamin B_6 and the methods of its preparation in a concentrated state. Biochem. J., vol. 30, p. 304.

BURR, G. O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., vol. 82, p. 345.

DEAN, H. K. 1938 Utilization of fats. 306 pp. illus. Chemical Publishing Co. of N. Y., Inc., New York.

HILDITCH, T. P. 1940 The chemical constitution of natural fats. 438 pp., illus. John Wiley and Sons, Inc., New York.

QUACKENBUSH, F. W., B. R. PLATZ AND H. STEENBOCK 1939 Rat acrodynia and the essential fatty acids. J. Nutrition, vol. 17, p. 115.

QUACKENBUSH, F. W., H. STEENBOCK, F. A. KUMMEROW AND B. R. PLATZ 1942 Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis. J. Nutrition, vol. 24, p. 225.

RICHARDSON, L. R., AND A. G. HOGAN 1936 Skin lesions in the rat associated with the vitamin B complex. Mo. Agr. Exp. Sta. Res. Bull. no. 241.

ROBERTS, W. L. AND H. A. SCHUETTE 1934a Characteristics and composition of Wisconsin-grown tobacco seed oil. J. Am. Chem. Soc., vol. 56, p. 207.

SCHUETTE, H. A., M. A. COWLEY AND C. Y. CHANG, 1934b The characteristics and composition of coffee bean oil. J. Am. Chem. Soc., vol. 56, p. 2085.

SCHUETTE, H. A., M. A. COWLEY, H. A. VOGEL AND M. M. MUELLER 1940b Fenugreek seed oil. Oil and Soap, vol. 17, p. 122.

SCHUETTE, H. A., AND J. A. KORTH 1940c The seed oil of the highbush cranberry. Oil and Soap, vol. 17, p. 265.

SCHUETTE, H. A., R. W. THOMAS AND M. V. DUTHEY 1930 Brazil nut oil. J. Am. Chem. Soc., vol. 52, p. 4114.

SCHUETTE, H. A., AND R. G. ZEHNPFENNIG 1937 The seed oil of the hackberry. Oil and Soap, vol. 14, p. 269.

STOUT, A. W., AND H. A. SCHUETTE 1932 Rye germ oil. J. Am. Chem. Soc., vol. 54, p. 3298.

ZEHNPFENNIG, R. G., AND H. A. SCHUETTE 1941 Elm seed oil. II. Studies on fatty acid separation. Oil and Soap, vol. 18, p. 189.

THE UTILIZATION OF *d*-GLUCONO-δ-LACTONE BY THE ORGANISM OF THE YOUNG WHITE RAT

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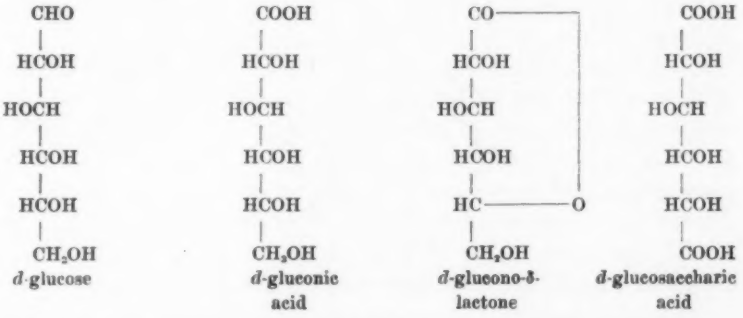
(Received for publication March 15, 1943)

d-Glucose is readily oxidized to *d*-gluconic acid by bromine in acid or neutral solution. Gluconic acid has also been isolated as a product of the oxidation of glucose by the enzyme glucose dehydrogenase of the liver (Harrison, '32). The occurrence of such a reaction in the intact living organism has, however, not yet been proven. Gluconic acid in aqueous solution forms an equilibrium mixture with its anhydrides, the γ- and δ-lactones. An oxidation product of gluconic acid is glucosaccharic acid.



Little information is available as to the utilization of gluconic acid (or its lactones) in the animal organism. The evidence suggests the following metabolic changes: (1) Part of the administered gluconic acid may be oxidized to *d*-gluco-

saccharic acid, which has been isolated from the urine. The remainder is believed to undergo complete oxidation (Mayer, '02). (2) Part of the gluconic acid may be excreted in the urine unchanged (Baer and Blum, '11; Hermann, '30; Paderi, '15; and Schott, '11). (3) Part of the acid may be converted to glucose as evidenced by the formation of glucose when gluconic acid is incubated with liver tissue, and by an increased content of liver glycogen when the acid is fed to rabbits (Paderi, '15).

Gluconic acid may be converted to pentose in the laboratory (Ruff, 1899). A similar reaction in vivo has been suggested as an explanation of the origin of the pentose excreted in pentosuria, i. e., an abnormal oxidation reaction of glucose in this error of metabolism. It is also possible that gluconic acid may be an oxidation product of glucose which, in the normal organism, is further oxidized to carbon dioxide and water, but which, in the pentosuric, escapes further oxidation and is excreted as pentose. However, neither Salkowski (1899) nor Mayer ('02) could detect pentose in the urine of rabbits to which gluconic acid was fed.

Baumgarten ('05–'06) reported that gluconic acid was readily oxidized by both the normal and the depancreatized dog and also by the human diabetic, an observation which was not confirmed with rabbits by Hermann ('30). That gluconic acid was excreted largely unchanged has been assumed in studies of the value of gluconic acid as an acidifying agent for human urine (Gold and Civin, '39). When considerable amounts of the δ-lactone were fed to man under controlled experimental conditions, the urine became more acid. The acidifying value of the lactone under the usual conditions of diet and fluid intake was, however, believed to be slight.

The experimental studies have been limited by the lack of accurate quantitative methods for the determination of gluconic acid and its possible degradation products in tissues and biological fluids. Attempts have usually been made to isolate gluconic acid and glucosaccharic acid and their de-

rivatives by procedures which are far from giving quantitative results.

Our interest in gluconic acid was stimulated by recent industrial developments which have made the acid available commercially at a reasonable cost. Calcium gluconate has been used in therapeutics as an organic salt of calcium. Gluconic acid,[1] its lactone and salts, have many possible applications in the food industries. The lactone in aqueous solution forms an equilibrium mixture with the acid. At room temperature, after approximately 90 minutes, the solution contains about 20% of the lactone and 80% of the acid.

A direct method of study of the utilization of gluconic acid or its lactones, namely, the study of its utilization as a source of energy in the intact organism, has not been employed, so far as is known to us. If gluconic acid is oxidized to any significant extent, it should be able to furnish calories and thus to promote the growth of young white rats when added as a supplement to a diet whose essential deficiency is with respect to inadequate calorific value (Bendaña and Lewis, '35). The experiments detailed in the present paper represent such a study of the biological value of glucono-δ-lactone in the young white rat. Our results show that the addition of the lactone to the low-calorie diet was followed by an increased rate of growth which was similar to that observed when isocaloric amounts of a readily utilizable carbohydrate, glucose, served as the source of supplementary calories. The amount of the lactone which could be administered and utilized, however, was limited by its irritant action in the gastrointestinal tract, which resulted in diarrhea, as already observed by others (Mitchell, Cook and O'Brien, '39; Paderi, '15).

EXPERIMENTAL

Young white rats in litter units were used for the experiments. After weaning, they received a stock diet of milk,

[1] We are indebted to Charles Pfizer and Company of New York, who generously placed at our disposal the δ-lactone of gluconic acid used in these experiments.

whole wheat bread, and lettuce, or of a commercial rat chow. When the rats reached the weight of 65 to 80 gm., they were fed a basal diet low in calorific value (approximately 14.0 Cal. daily) in the restricted amounts shown in table 1. In most cases this diet permitted slight growth of rats of the weight used. Extra calories in the form of glucose monohydrate or glucono-δ-lactone were added as a supplement to the basal diet, and the effects on growth were observed. The various

TABLE 1

Basal and experimental diets.

	1	2	3
	gm.	*gm.*	*gm.*
Casein	1.2	1.2	1.2
Cod liver oil	0.1	0.1	0.1
Lard	0.1	0.1	0.1
Agar	0.1	0.1	0.1
Salt mixture [1]	0.4	0.4	0.4
Glucose	1.5	2.25	1.5
Glucono-δ-lactone			0.75
Daily food intake [2]	3.4	4.15	4.15
Daily calorie intake (calculated) [3]	14.0	17.0	16.6

[1] Osborne-Mendel salt mixture to which copper sulfate has been added to give a concentration of approximately 0.04% of copper.

[2] Each rat also received one dried yeast tablet daily (appproximately 400 mg.).

[3] Calculated on the basis of 3.5 cal. per gram of glucono-δ-lactone and of 1.4 cal. per yeast tablet.

diets are given in table 1. Although glucose was fed as the commercially available monohydrate, the equivalent amount of anhydrous glucose is presented in the diets as given in table 1. One gram of anhydrous glucose is equivalent to 1.1 gm. of the monohydrate. The calorific value of the lactone was stated by the manufacturers to be 3.5 Cal. per gram (Committee on Foods, '33). The extra calories of the supplementary glucose and gluconolactone were 3.0 and 2.6, respectively, amounts somewhat over 15% of the calorific value of the basal diet. In earlier experiments, an attempt was

made to add, as a supplement, 1.5 gm. of the gluconolactone daily. This resulted in a rather severe diarrhea, so that the amount of lactone was reduced to 0.75 gm. (diet 3). With this diet, no diarrhea of significance was observed.

All rats were maintained on the low-calorie basal diet for 6 to 7 days. At the end of this period, the rats of each litter were divided into three groups. The first group continued to receive the basal diet (diet 1); the second received the basal diet plus added glucose (diet 2); the third received the basal diet plus gluconolactone (diet 3). The animals were weighed every 3 days. Since the animals had similar nutritional histories at the beginning of the experiment, the average gains for the early periods seemed more significant, particularly since, as the animals gained in weight, the calorific value of the diet would be expected to be less adequate. The results of a period of 2 weeks (litter A) and 3 weeks (litter B) are summarized in table 2.

The results presented in table 2 show that the gluconolactone was almost as effective in the promotion of growth as was glucose. Thus with litter A, the average daily gains with supplementary glucose and gluconolactone were 208 and 213%, respectively, of that of the control group (basal diet); with litter B, the corresponding values were 217 and 191%.

Further evidence of the utilization of the gluconolactone is afforded by table 3. After the initial 2 or 3 week period, the diets were changed. The animals which had received the control diet (diet 1) were fed the gluconolactone supplement (diet 3), and those which had received extra calories as glucose (diet 2) or the lactone (diet 3) were placed on the control diet. The responses to these changes in diet were striking. The animals which had previously shown fair or good growth on diets 2 or 3 ceased to gain significantly and, for the most part, lost weight slightly. This loss in weight was not unexpected, since the basal diet supplied the minimal calories for the animals at the beginning of the experiments and could hardly be expected still to be adequate for the animals after fair growth had proceeded for 2 or 3 weeks. On the

other hand, the animals previously fed the basal diet grew satisfactorily when placed on the diet containing supplementary gluconolactone. After 2 weeks (period II) on the basal

TABLE 2

Growth of young white rats fed low-caloric diets with and without supplements.

Weights of rats fed low calorie diets and similar diets in which extra calories as glucose or gluconolactone were provided.

The feeding periods were 14 and 21 days for litters A and B, respectively.

RAT NO.	SEX	DIET	WEIGHT		CHANGE IN WEIGHT	TOTAL GAIN FOR GROUP	AVERAGE DAILY GAIN PER RAT
			Initial	Final			
			gm.	*gm.*	*gm.*	*gm.*	*gm.*
			Litter A				
11	F	1	82.9	87.6	+ 4.7		
16	F	1	64.6	72.2	+ 7.6	27.5	(0.65)
18	F	1	64.1	79.3	+15.2		
12	F	2	71.6	87.9	+16.3		
13	F	2	76.9	92.0	+15.1	56.7	(1.35)
19	M	2	69.1	94.4	+25.3		
14	F	3	73.5	90.0	+17.4		
15	M	3	70.0	95.2	+25.2	58.3	(1.39)
17	F	3	67.8	83.5	+15.7		
			Litter B				
22	F	1	70.6	82.4	+11.8		
23	M	1	69.5	76.6	+ 7.1	52.7	(0.62)
25	F	1	66.2	82.1	+15.9		
30	F	1	62.0	79.9	+17.9		
26	M	2	62.0	95.0	+33.0		
27	M	2	62.9	90.3	+27.4	113.6	(1.35)
29	F	2	61.4	83.5	+22.1		
31	M	2	71.2	102.3	+31.1		
20	M	3	74.4	91.4	+17.0		
21	M	3	71.5	97.6	+26.1		
24	M	3	67.7	98.8	+31.1	99.4	(1.18)
28	F	3	64.4	89.6	+25.2		

diet, two groups of litter A were again fed the supplemented basal diets (diets 2 and 3). Growth was resumed, although, as anticipated, the rate was not as great with these now heavier animals.

Two of the rats of group 3 of litter B were placed on diet 4 after the initial period on diet 3. This diet resembled diet 1 but contained gluconolactone instead of glucose. Glucono-

TABLE 3

Changes in weight of rats on low-caloric diets and diets supplemented with glucose and gluconolactone.

The values presented represent changes in weight on the various diets indicated. The periods are of 2 weeks' duration, except for period I with litter B, which extended for 3 weeks.

RAT NO.	PERIOD I		PERIOD II		PERIOD III	
	Diet	Weight change	Diet	Weight change	Diet	Weight change
		gm.		gm.		gm.
			Litter A			
11	1	+ 4.7	3	+21.0		
16	1	+ 7.6	3	+13.4		
18	1	+15.2	3	+16.5		
12	2	+16.3	1	+ 8.1	3	+13.3
13	2	+15.1	1	— 3.8	3	+14.2
19	2	+25.3	1	— 2.4	3	+13.0
14	3	+17.4	1	— 3.2	2	+ 9.9
15	3	+25.2	1	— 0.1	2	+12.4
17	3	+15.7	1	— 5.5	2	+10.1
			Litter B[1]			
22	1	+11.8	3	+19.8		
23	1	+ 7.1	3	+13.5		
25	1	+15.9	3	+ 6.2		
30	1	+17.9	3	+15.2		
26	2	+33.0	1	— 2.6		
27	2	+27.4	1	— 0.7		
29	2	+22.1	1	+ 3.2		
31	2	+31.1	1	+ 2.2		
20	3	+17.0	2	+14.1		
21	3	+26.1	4[2]	— 3.6		
24	3	+31.1	4[2]	— 7.1		
28	3	+25.2	1	— 4.8		

[1] Period I is of 3 weeks' duration and period II of 2 weeks' duration, in the experiments with litter B.

[2] Diet 4 is the same as diet 1 except that 1.5 gm. of the gluconolactone replaces the glucose (1.5 gm.). The caloric values of diets 1 and 4 are essentially the same.

lactone was the only available "carbohydrate" except for the carbohydrate present in the yeast tablet. These two animals showed, as expected, prompt loss of weight, although in one case the loss of weight was no greater than the loss of an animal of the same group which was fed the basal diet (rat 28).

We believe that the data here presented and similar data obtained by us indicate clearly that in small quantities the gluconolactone can be utilized for growth almost as efficiently as glucose. Since the limiting factor in the basal diets was the low caloric value, and since the addition of glucose or the gluconolactone increased the rate of growth significantly, it seems logical to conclude that the gluconolactone served as a source of energy either by conversion to glucose and oxidation of the glucose thus formed, or by direct oxidation. Experiments are planned to test further the fate of the gluconolactone in the animal organism. The value of gluconolactone in nutrition would seem to be limited definitely by its laxative action in the gastrointestinal tract (Mitchell, Cook and O'Brien, '39).

SUMMARY

Young white rats were fed a diet whose limiting factor was an inadequate caloric value. When this basal diet was supplemented by either glucose or *d*-glucono-δ-lactone as a source of additional calories, growth at a rate definitely greater than that of animals fed the basal low-caloric diet was observed. Glucose and the gluconolactone were almost equally effective in the promotion of growth. These studies are believed to indicate that under the conditions of the present experiments, gluconolactone in limited amounts may serve as a source of energy, utilization being effected either by direct oxidation or by conversion to glucose and oxidation of the latter.

LITERATURE CITED

BAER, J., AND L. BLUM 1911 Über die Einwirkung chemischer Substanzen auf die Zuckerausscheidung und die Acidose. III. Arch. exp. Path. Pharmakol., vol. 65, pp. 1–34.

BAUMGARTEN, O. 1905-6 Ein Beitrag zur Kenntniss des Diabetes mellitus. Ztschr. exp. Path. Therap., vol. 2, pp. 53–72.

BENDAÑA, A., AND H. B. LEWIS 1935 The utilization of inulin for growth by the young white rat. J. Nutrition, vol. 10, pp. 507–515.

COMMITTEE ON FOODS 1933 Pfizer Glucono-Delta-Lactone. J. Am. Med. Assn., vol. 100, p. 577.

GOLD, H., AND H. CIVIN 1939 Gluconic acid as a urinary acidifying agent in man. J. Lab. Clin. Med., vol. 24, pp. 1139-1146.

HARRISON, D. C. 1932 The product of the oxidation of glucose by glucose dehydrogenase. Biochem. J., vol. 26, pp. 1295–1299.

HERMANN, S. 1930 Zur Pharmakologie der Gluconsäure III. Arch. exp. Path. Pharmakol., vol. 154, pp. 175–192.

MAYER, P. 1902 Experimentelle Untersuchungen über Kohlenhydratsäuren. Ztschr. klin. Med., vol. 47, pp. 68–108.

MITCHELL, H. S., G. M. COOK AND K. L. O'BRIEN 1939 Effect of several calcium salts on the utilization of lactose. J. Nutrition, vol. 18, pp. 319–327.

PADERI, C. 1915 Sur le mode de se comporter de l'acide glyconique dans l'organisme. Arch. ital. biol., vol. 64, pp. 81–88.

RUFF, O. 1899 Über die Verwandlung der d-Gluconsäure in d-Arabinose. Ber. deutsch. chem. Gesellsch., vol. 31, pp. 1573–1577.

SALKOWSKI, E. 1899 Über das Vorkommen von Pentosen im Harn. Ztschr. physiol. Chem., vol. 27, pp. 507–539.

SCHOTT, E. 1911 Über Verhalten von Glykonsäure und Zuckersäure im Organismus. Arch. exp. Path. Pharmakol., vol. 65, pp. 35–37.

THE EFFECT OF COMMERCIAL CLARIFICATION ON THE VITAMIN CONTENT OF HONEY [1] [2]

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(Received for publication March 19, 1943)

In a previous paper (Haydak and co-workers, '42) we reported the vitamin content of clarified and unclarified honeys. It subsequently developed that the clarification was done under laboratory conditions and the question was raised whether commercial processing of this type would give the same reductions in vitamin content. In order to obtain data on this question, samples of unclarified and clarified honey were obtained from two commercial packers.

The methods used were: for thiamine, that of Hennessy and Cerecedo ('39); for riboflavin, that of Snell and Strong ('39); for ascorbic acid, Bessey ('38); pantothenic acid Strong, Feeney and Earle ('41), and for nicotinic acid, the method of Melnick and Field ('40). We also determined the anti-hemorrhagic activity of clarified and unclarified honeys, following method of Almquist ('41) as modified slightly by Vivino and co-workers ('43).

The results of the determinations are presented in table 1, in comparison with those previously published. From the table it is evident that there was a general decline in the concentration of vitamins due to the commercial clarification, although generally to a lesser degree than in the laboratory processed sample.

[1] Paper no. 2075, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

[2] The completion of this work was made possible through the research grant from W. F. Straub & Co., Chicago, Illinois.

The prothrombin time was 3′ 55″ and 4′ 11″; 4′ 19″ and 4′ 07″ for unclarified and clarified honeys, respectively, when 2 gm. of honey were supplied daily for 4 days during the curative period to vitamin K depleted chicks. This corresponds closely with the average prothrombin time of 4′ 02″ for the positive controls which received 0.5 μg. of synthetic vitamin K (2-methyl–1, 4–naphthoquinone in ethyl laurate) daily during the same period. The prothrombin time of the negative controls was 25 minutes. It is apparent that clarification did not perceptibly affect the antihemorrhagic activity of honey. This fact would suggest that this activity is not due to the pollen present in honey but to substances dissolved in it.

TABLE 1

Vitamin content per 100 gm. of clarified and unclarified honey.

SAMPLE	THIAMINE	RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID	ASCORBIC ACID
	μg.	*μg.*	*μg.*	*μg.*	*mg.*
(a) Unclarified	5.1	80.0	100.0	400.0	2.1
(b) Clarified	3.6	62.0	92.0	290.0	1.9
Decrease %	*30.0*	*22.5*	*8.0*	*27.5*	*9.5*
(a) Unclarified	5.9	61.0	81.0	720.0	2.0
(b) Clarified	4.3	33.0	63.0	610.0	1.6
Decrease %	*27.1*	*45.9*	*22.2*	*15.3*	*20.0*
Previous results, laboratory process					
(a) Unclarified	8.4	93.0	176.0	870.0	2.7
(b) Clarified	5.4	64.0	97.0	460.0	1.9
Decrease %	*35.7*	*31.2*	*44.9*	*47.1*	*29.6*

SUMMARY AND CONCLUSIONS

Two pairs of samples of clarified and unclarified honey were assayed for thiamine, riboflavin, pantothenic acid, nicotinic acid and ascorbic acid contents. There was a general decrease in the vitamin concentration of the clarified samples, ranging from 8 to 45%. There was no perceptible reduction in the antihemorrhagic activity of the clarified honeys compared with the unclarified controls.

ACKNOWLEDGMENT

The authors are indebted to Dr. H. J. Sloan for allowing the use of chicks and equipment for the vitamin K assay.

LITERATURE CITED

ALMQUIST, H. J. 1941 Report on vitamin K assay by curative biological test. J. Ass. Offic. Agric. Chem., vol. 24, pp. 405–413.

BESSEY, O. 1938 A method for the determination of small quantities of ascorbic acid and dehyroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem., vol. 126, pp. 771–784.

HAYDAK, M. H., L. S. PALMER, M. C. TANQUARY AND A. E. VIVINO 1942 Vitamin content of honeys. J. Nutrition, vol 23, pp. 581–588.

HENNESSY, D. J., AND L. R. CERECEDO 1939 The determination of free and phosphorylated thiamine by a modified thiochrome assay. J. Amer. Chem. Soc., vol. 61, pp. 179–183.

MELNICK, D., AND H. FIELD, JR. 1940 Chemical determination of nicotinic acid: inhibitory effect of cyanogen bromide upon the aniline side reactions. J. Biol. Chem., vol. 135, pp. 53–58.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. J. Ind. Eng. Chem. (anal.), vol. 11, pp. 346–350.

STRONG, F. M., R. E. FEENEY AND A. EARLE 1941 Microbiological assay for pantothenic acid. J. Ind. Eng. Chem. (anal.), vol. 13, pp. 566–570.

VIVINO, A. E., M. H. HAYDAK, L. S. PALMER AND M. C. TANQUARY 1943 Anti-hemorrhagic vitamin effect of honey. Proc. Soc. Exp. Biol. Med., vol. 53, pp. 9–11.

EFFECT OF INCREASING CALCIUM CONTENT OF DIET UPON RATE OF GROWTH AND LENGTH OF LIFE OF UNMATED FEMALES

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(Received for publication March 31, 1943)

In previous work (Sherman and Campbell, '35) the calcium content of a diet which had been proven to be adequate for growth and reproduction generation after generation was increased by the addition of calcium carbonate from the initial level of 0.20% to a level of 0.34% of calcium in the air-dry food. This resulted in (1) a very slightly increased growth; (2) no significant difference in adult size but a higher level of adult vitality; and, (3) in the males, an increased length of life. The effect upon the length of life of the females was, however, somewhat in doubt. The experimental animals (rats) had been mated early and allowed to breed as freely as possible. The females which received the diet containing the larger amount of calcium reared more young and brought them to a higher average weight at a standard weaning age. When, then, these females lived only insignificantly longer than those on the diet of lower calcium content, the question arose whether this was because the females either were less able than the males to make full use of the extra food calcium, or had more ways in which to invest it. Was it because their extra food calcium had been invested in the bearing and suckling of more young that the lives of the mothers were not more distinctly prolonged?

The present paper reports the results of experiments designed to ascertain whether females not called upon to furnish

calcium to offspring would gain in longevity through the same increase of calcium intake.

EXPERIMENTAL

Equal numbers of female rats of the same hereditary and nutritional background were fed the same diets 16 and 162 as described in the paper above noted. The initial age was 28 days, and each experimental animal was matched by a littermate sister, the one receiving diet 16 with 0.20%, and the other diet 162 with 0.34%, of calcium. All of these experimental animals were observed daily and weighed weekly throughout the complete life span. The data of early growth and length of life are summarized in table 1.

TABLE 1

Records of unmated female rats on diets of different calcium contents.

CATEGORY OF INTEREST	ON DIET 16. 0.20% CALCIUM		ON DIET 162. 0.34% CALCIUM	
	No. of cases	Mean ± P.E. [1]	No. of cases	Mean ± P.E. [1]
Gain in weight, 5th to 8th weeks *(gm.)*	51	46.9 ± 0.83	51	58.4 ± 0.69
Age at death *(days)*	51	795 ± 16.	51	858 ± 12.

[1] This precision measure is the classical probable error of the mean.

DISCUSSION

As in the experiment previously mentioned, growth was slightly more rapid in the animals receiving the higher calcium intake. The noticeable difference was that in the unmated females, the increased calcium intake resulted in an extension of the life cycle by an average of 63 days or 8%, or in essentially equal proportion to the gain shown by the males in the experiments of the previous series above mentioned. The significance of the increase in length of life is further shown by the fact that the proportion of cases living to 900 days and over was 45% among those of higher, against 31% among those of lower, calcium intake.

These findings gain added interest when considered in connection with those of a related investigation in this laboratory in which it was found that mated females receiving food of still higher calcium content both raised more young and enjoyed an increased length of life (Van Duyne, Lanford, Toepfer, and Sherman, '41).

CONCLUSION

A limited addition of calcium to a diet of about minimal adequate calcium content results in equally increased length of life in comparable male and female rats if the latter do not invest the extra calcium in increased reproduction and lactation instead.

LITERATURE CITED

SHERMAN, H. C., AND H. L. CAMPBELL 1935 Effects of increasing the calcium content of a diet in which calcium is one of the limiting factors. J. Nutrition, vol. 10, p. 363.

VAN DUYNE, F. O., C. S. LANFORD, E. W. TOEPFER AND H. C. SHERMAN 1941 Lifetime experiments upon the problem of optimal calcium intake. J. Nutrition, vol. 21, p. 221.

MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Announcement is made that Mead Johnson and Company has renewed the arrangements for a period of 5 years whereby the annual award of $1,000.00 will be given for research dealing with the vitamin B complex. The recipient of this award will be chosen by a committee of judges of the American Institute of Nutrition. Nominations are solicited for the 1944 Award.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calender year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1943 must be in the hands of the Secretary by January 10, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

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